PROGRESS IN SPORT FISHERY RESEARCH 1969

United States Department of the Interior
Fish and Wildlife Service
Bureau of Sport Fisheries and Wildlife

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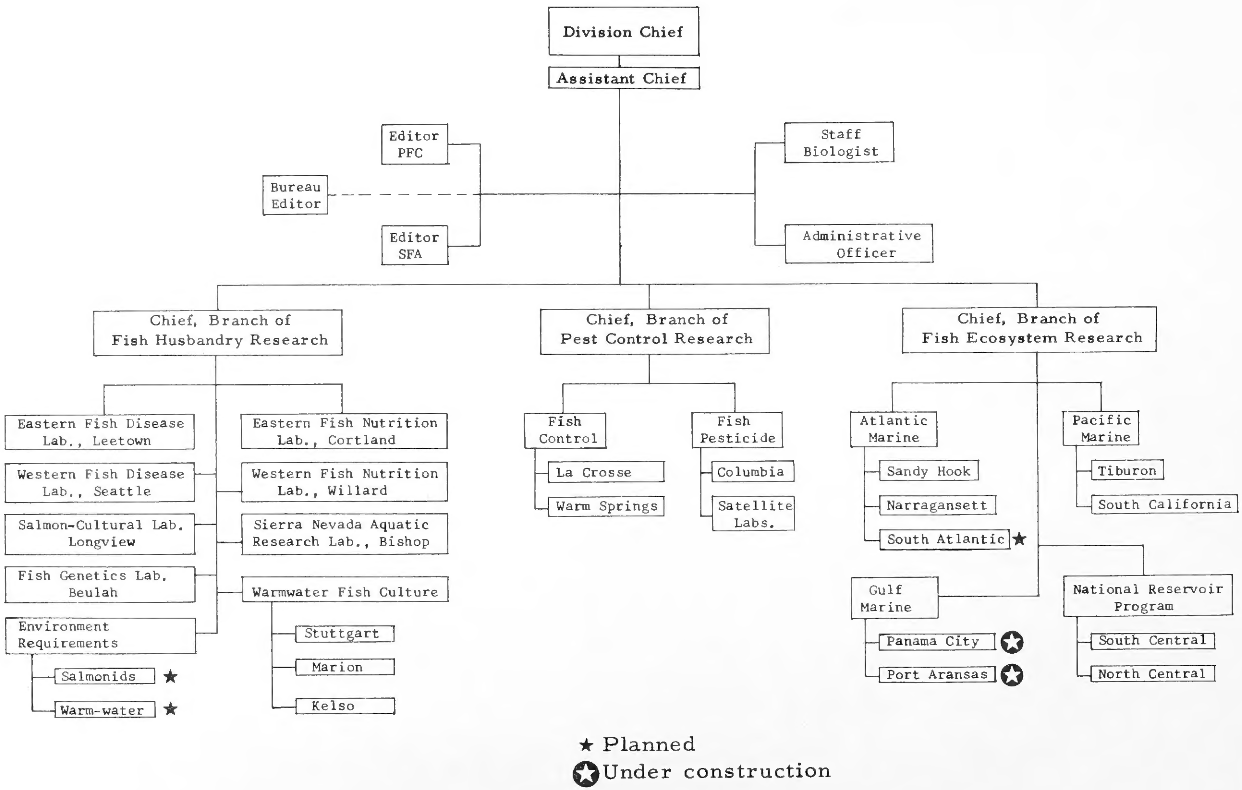
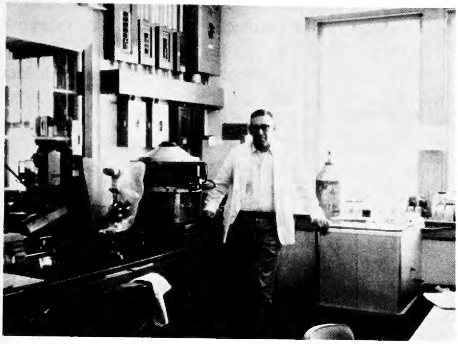


FOREWORD

SOMEONE HAS SAID that research is a small part inspiration, a large part perspiration. The inspiration part is by far the more important, of course. Sometimes it comes from an example and that is the purpose of the true story which follows.

Early in 1969, fishery research lost a man of great character and promise when Clarence E. (Ed) Dunbar died suddenly at Leetown, West Virginia. Almost 20 years ago Ed joined the staff of the Leetown Laboratory as a fish culturist at $2,450 a year to care for the trout used in disease research. He was about 34 years old then, married and with two children, and with something less than a high school education.



By 1969, Ed had a high school diploma, a college degree. He was a professional histopathologist occupying a key position on the Leetown staff. He had had special courses in histochemistry at Kansas University and pathology at Ohio State. He had presented papers at technical meetings, published in his specialty, and was respected as an authority. He taught in the Leetown fish disease courses, and gave demonstrations to visiting scientists from all over the world.

From a background in West Virginia coal mining to histopathology in a West Virginia research laboratory took personal career development quite beyond the vision of most people. Ed completed high school graduation requirements, attended classes and studied on his own time until he received his Bachelor's degree, all the while perfecting laboratory techniques and proficiency in separating normal from pathological tissues. How in the world did he do it? Of course he had the support and encouragement of his wife, the sympathetic urging of the laboratory director and his co-workers. But, in the final analysis, it was his own perseverance, personal sacrifices, and ambition to excel in a field of research he had grown to love and respect.

Ed's death has left a large and painful vacancy in the Leetown Laboratory staff, but his life example has left so much more that should encourage, motivate, and, yes, inspire those who otherwise may feel the road up is too steep, or the toll too costly. It has been done--it can be done. That is Ed Dunbar's memorial and legacy.

Paul E. Thompson, Chief
Division of Fishery Research

PEST CONTROL AND THE AQUATIC ECOSYSTEM

The value of the sport fishery depends on the quality and quantity of fish taken per unit of fishing effort. Fish management requires thorough knowledge of the life history of each fish, environmental requirements (and tolerances), relations to other species (predator and prey), analysis of habitat, and methods to manipulate and control populations. Ecological evaluations of the kinetics of energy flow through food chains and greater efficiency in producing sport fishes from available energy sources is the key to management success. Thus, as we become committed to "priorities for the Seventies," our contribution to society will come through effective research leading to good stewardship and wise resource use by improving and maintaining biological systems in aquatic environments.

In the past, we gave high priority to study of the persistent organochlorine insecticides because of their acute toxicity, accumulation in food chain organisms, and chronic effects. Typically, insecticides in the aquatic ecosystem cause acute toxicity to both fish and aquatic invertebrates. Resistant individuals that survive accumulate and transfer pesticide residues to other members of the ecosystem and to man. The degradation of the value and production of favored fish is often subtle and unnoticed. More recently, our attention has turned to the organophosphorous and carbamate insecticides. We are concerned about their interaction with organochlorine pesticides and those compounds commonly called PCBs (polychlorinated biphenyls). Herbicides rate more attention since the controversy on 2,4,5-T and the report that its contaminant dioxin induces abnormal fetuses in special strains of mice. Because some phenoxy herbicides are applied directly in water for control of aquatic plants to enhance fish production and sport fishing, our investigations center on the fate of herbicide residues and effects on fish, fish-food organisms, and other aquatic organisms.

Excessive aquatic weed growth is a universal management problem. It interferes with fish culture and angling. The relation of aquatic flora to a fishery is often poorly understood and documented even in the most technically detailed studies. Generally, most research on aquatic weeds and their control has been focused on chemical, mechanical, or biological removal. Very little effort, however, has been made to understand the flow of plant nutrients and energy in the aquatic ecosystem and how to manipulate producer and food chain organisms to maximize the harvestable yield. Thus, our concern is with aquatic ecology, plant and animal physiology, kinetics of plant nutrients in water and soil chemistry, pesticide toxicology, nutritional biochemistry, life history of the aquatic food chain species (consumer organisms), and dependent relations with aquatic plants and algae (producer organisms). We know too little about the flow of energy through the food chain to fish, the selectivity or preferences of fish for different food organisms and aquatic plants, and the interdependence of plants and food organisms.

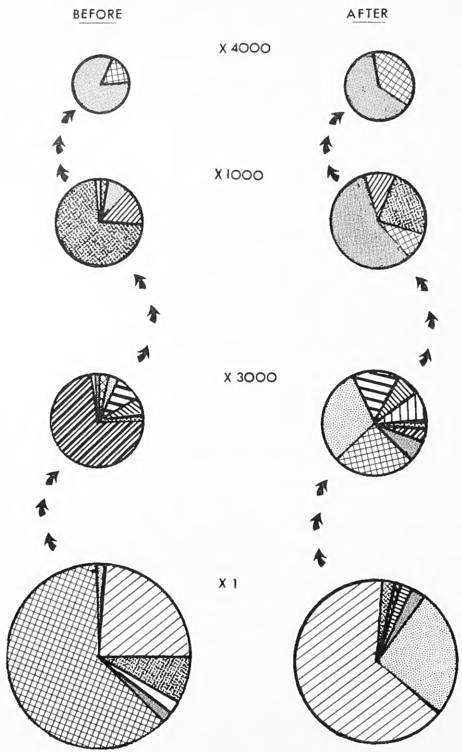
We have examined certain pesticides and their effects on the aquatic ecosystem in relation to maximizing production of sport fish populations. Antimycin, a short-lived and non-residue producing chemical, has been used effectively to alter the structure of bass-bluegill populations. This selective pesticide thinned out the stunted bluegill and reinstituted a desirable predator-prey relation to improve angling quality. This chemical affects only the secondary and tertiary consumers. Toxaphene, in contrast, has been used in a similar manner except that the primary consumers (fish-food organisms) are also destroyed. Toxaphene lingers--the residues are biologically transferred up the food chain and accumulate in tissues of fish and other predators. Herbicides are generally short-lived but have a much more subtle effect on the aquatic ecosystem (Figure 1). The primary producer organisms are directly affected. This is the objective of the management biologists, but the changes induced are transferred all the way up the food chain and dramatically alter the flow of energy. In this example, sodium endothal is selectively toxic to certain submersed rooted plants and eliminates them from the habitat--releases these stored nutrients and energy to decomposer organisms (bacteria, etc.) which in turn feed diatoms, rotifers, protozoans, etc. This also changes some of the physical features of the habitat--weed clinging insect larvae and protective cover for the invertebrates and small fish are now more vulnerable to predation. Turbidity from the plankton is sharply increased but does not adversely affect feeding by predatory fishes at the secondary and tertiary trophic level. The net result is a more efficient system for benefiting the desirable sport fishes. Removal of excessive plant growth redirects energy flow, improves fish growth rates, and increases in production, and catch per unit effort is evidence of improvement in the sport fishery.

Adequate labeling, recommendations, and guidelines for safe and effective use of pesticides require appropriate data on toxicity, efficacy, and residues under different conditions. Research for evaluation of weed control chemicals must develop the following information: 1) toxicity to the target species; 2) relative toxicity to non-target species of plants, invertebrates, fish, other aquatic animals, birds, mammals, and man; 3) fate of residues and significance in water, fish, crops, livestock and other foods of man; 4) conditions affecting toxicity, efficacy and persistence of residues in the proposed pattern of use, e. g., water chemistry, temperature, variations in susceptibility of species at various life stages and seasons, inflow dilution, contact time, rate of degradation, deactivation or detoxification; 5) potentiating or synergizing activity of carriers, formulations, or combination with other contaminants and pesticides, metabolites and degradation products.

An orderly system of toxicological screening and evaluation is required. Pesticides may be applied in both standing and flowing situations, and the bioassay methods must be sufficient to measure the herbicide concentration and the contact time necessary for aquatic plant control or toxicological effects on other aquatic organisms. Thus, our research includes static, intermittent-flow or constant-flow bioassay systems depending on the length of the testing and investigator's desire to most nearly simulate the lentic or lotic environmental conditions. Temperature, biomass-volume rates, water chemistry and light intensity or periodicity are also important considerations with regard to the reaction of aquatic organisms or plants to the chemicals.

Economical considerations are dictated by the effectiveness of the chemical in channelization of energy to increased quality or quantity of the harvestable yield. Thus, our research method for evaluation of the cost-benefit aspects of pesticide use must include analysis of energy flow limiting factors, and biomass produced at each trophic level with respect to the biotic potential of the species under management.

Charles R. Walker, Chief
Branch of Pest Control Research



TROPHIC LEVELS (and organisms)	BEFORE g/m^2	BEFORE Cal/cm^2	AFTER g/m^2	AFTER Cal/cm^2
Tertiary Consumers				
Lmb (Largemouth bass > 3")	0.067	0.0403	0.113	0.0680
Bg (Bluegill > 3")	0.046	0.0277	0.024	0.0144
Total production	0.113	0.0680	0.137	0.0824
Secondary Consumers				
Lmb (Largemouth bass < 3")	0.114	0.0685	0.600	0.306
Bg (Bluegill < 3")	0.493	0.2910	4.020	2.060
Dgf (Dragon fly)	4.240	2.1300	1.800	0.920
Dsf (Damsel fly)	0.850	0.4200	0.790	0.405
Cdf (Caddis fly)	0.090	0.0045	--	--
Total production	5.787	2.9140	7.210	3.691
Primary Consumers				
An (Annelids)	0.180	0.00064	0.857	0.03680
Mgf (Midge larvae)	0.060	0.00027	0.714	0.03060
Tbl (Tabanid larvae)	0.240	0.00108	0.423	0.01820
Msq (Mosquito larvae)	tr.	tr.	--	--
Pml (Phantom midge larvae)	--	--	0.238	0.01023
Bml (Biting midge larvae)	tr.	tr.	0.1595	0.00068
Wb (Water beetle)	tr.	tr.	0.0317	0.00136
Tdp (Tadpoles)	0.168	0.10180	0.1751	0.00840
Sn (Snails)	1.920	0.00864	0.1955	0.00840
Zpk (Zooplankton)	0.060	0.00026	0.1862	0.00800
Total production	2.630	0.11309	2.8800	0.12217
Primary Producers				
Ctt (Cattail)	853.0	299.00	2359.0	847.00
Ndr (Needle rush)	tr.	tr.	14.9	5.39
Cnt (Coontail)	2041.0	714.35	--	--
Bpw (Bushy pondweed)	36.2	13.00	--	--
Mkg (Muskgrass)	tr.	tr.	98.5	28.18
Sga (Spirogyra algae)	223.0	78.00	78.8	28.30
Cda (Cladophora algae)	--	--	30.0	11.00
Ppk (Phytoplankton)	20.3	7.14	878.0	192.40
Bpw (Broadleaf pondweed)	28.1	9.38	138.4	49.90
Production-sampling area	3201.9	1120.87	3577.6	1162.17
Total production	1236.0	430.38	1204.0	421.4
Total pond production - all levels	1244.53	433.475	1214.227	425.295

Figure 1:--Effects of a selective aquatic herbicide on the species composition and production of biomass in a pond ecosystem one week before and six weeks after 0.5 ppm of disodium endothall was applied during late summer to a 0.25 A pond.

FISH-PESTICIDE RESEARCH LABORATORY

Columbia, Missouri
Richard A. Schoettger, Director

HIGHLIGHTS

We completed 320 acute and continuous-flow bioassays of insecticides, herbicides, fungicides, fish eradicants, a rodenticide and eight PCBs (polychlorinated biphenyls) against fish.

In bioassays on many organophosphate insecticides now being suggested as alternatives to the more persistent pesticides, we find that they are particularly active where combined with other chemicals.

Dichlone was the most toxic of 16 herbicides tested against six species of crustacea.

Methoxychlor has an adverse effect on growth of adult cutthroat trout and ripening of females. Progeny of methoxychlor-treated fish are more resistant to the insecticide than progeny of controls.

Application rates of Dursban or malathion-parathion mixtures recommended for mosquito control are hazardous to fish and fish-food organisms.

Various crustaceans and immature aquatic insects exposed to 100 ng of DDT per liter accumulate residues of the insecticide that are 1,000 to 100,000 times greater than those in water.

Heptachlor stimulated growth of certain microorganisms, inhibited growth in others, but had no effect on gram-negative bacteria.

Rainbow trout exposed to dieldrin and subjected to forced swimming, preferentially utilize amino acids for energy, whereas DDT-treated and control fish use fats and carbohydrates.

The presence of DDT in a synthetic mixture of polychlorinated biphenyls (PCBs) was detected by use of a gas chromatograph-mass spectrometer.

Specific activity of acetyl cholinesterase in bluegill brain is correlated with water temperature.

Forced swimming and fasting of DDT-treated rainbow trout depleted their mesenteric fats, mobilized fat-stored DDT, and elevated DDT deposits in their brains. Death ensued and prior symptoms suggest DDT poisoning.

The uptake of 2,4-D dimethylamine salt from water by bluegills and channel catfish appears to be a more important source of 2,4-D residues than uptake from food.

Our analytical capabilities were extended to at least 20 other government agencies and included pesticide analyses of fish, water, sediments, and fish foods.

ACUTE TOXICITY OF PESTICIDES TO FISH

We completed 320 acute and chronic bioassays in spite of the loss of approximately 40,000 fishes resulting from two separate power failures, and finally, from the complete breakdown of the submersible well pump on December 5. Twenty-four-, 48-, and 96-hr TL_{50} values and corresponding 95 percent confidence intervals were calculated from tests conducted on toxicity of 60 pesticides to coho salmon, rainbow trout, channel catfish, bluegills, redear, and black crappie. The pesticides tested included insecticides, herbicides, fungicides, fish eradicants, a rodenticide, and polychlorinated biphenyl compounds (PCBs-industrial chemicals).

Many of the acute toxicity tests served as guidelines for further research. These include dieldrin and photo-dieldrin, Dyrene, Akton, malathion and its thionate analog, and parathion and its thioate analog. Also, many fishes were exposed to different pesticides in static and continuous-flow systems for use by the analytical section in perfecting their methods of residue analysis.

We obtained preliminary data on toxicity of several herbicides to bluegills. The 96-hour TL_{50} value for N,N-dimethyl-2,4-dichlorophenoxyacetamine was 0.9 mg/l and Igran was 2.7 mg/l, while Brush Rhap A-2D-2T, Ded Weed (2,4,5-T amine) and 2 A-D/2,4,5-T DMA gave 96-hour TL_{50} values of over 100 mg/l. Dipotassium endothall and Kling Tite 800 (naphthaleneacetic acid) were relatively non-toxic to coho salmon with 96-hour TL_{50} values of 100 and 75.7 mg/l, respectively. Weed and Brush-off 400 (diethylethanolamine salt) of two specific gravities (0.92 and 1.00) are relatively toxic to bluegills: 96-hour TL_{50} values for the 0.92 formulation was 0.16 mg/l, whereas the value of the other formulation was 0.28 mg/l. Two other herbicides, Veon and 2,4-D/2,4,5-T amine, gave 96-hour TL_{50} values of 40.9 and 22.6 mg/l, respectively.

Two PCBs were bioassayed for toxicity against channel catfish. The toxicity of Aroclor 1254 at 18 C. was 7.0 mg/l, whereas Aroclor 1248 was slightly more toxic. Definitive tests with 1254 and 1248 against bluegills gave 96-hour TL_{50} values of 2.7 and 0.3 mg/l, respectively. We also tested nine other PCBs, but because of failure in our water system these tests were discontinued after 24 hours. Little activity at either 24 or 48 hours for all 11 of these compounds was indicated. Consequently, these compounds should be tested in constant-flow systems longer than 96 hours to fully evaluate their toxicity to fishes.

We completed research on the toxicities of different pesticide combinations against rainbow trout and bluegills; a manuscript dealing with a portion of this work by Dr. Kenneth Macek is in review. General conclusions of this study were as follows: 1) in each case the results were the same whether the particular combination was tested against rainbow trout or bluegills; 2) malathion and copper sulfate interacted synergistically; 3) 18 combinations were additive; 4) DDT did not interact with any of the pesticides tested except BHC; 5) many organophosphorus insecticides being suggested as alternatives to the more persistent pesticides are particularly active when combined with other chemicals; and 6) several combinations currently recommended by the U. S. Department of Agriculture are synergistic in their toxicity to rainbow trout and bluegills (Table 1).

The influences of time and temperature on the toxicity of four pesticides to rainbow trout were established in static and continuous-flow bioassays. Preliminary tests against rainbow trout were completed under static conditions for dieldrin, DDT, Dursban, and Zectran. DDT, dieldrin, and Dursban were tested against rainbow trout under continuous-flow conditions. DDT exhibited a negative temperature coefficient of activity, being approximately twice as toxic at 4.4 C. as at 12.8 C. Dieldrin and Dursban both exhibited a positive temperature coefficient of activity. Dursban was three times as toxic at 12.8 C. than at 4.4 C. Dieldrin was only slightly more toxic at 12.8 C.

Several attempts were made to bioassay Guthion, carbaryl and DDT against various life stages of coho salmon, rainbow trout, channel catfish, bluegills, and black crappie to determine relative susceptibilities. As each lot of fish arrived at the laboratory, they were held until individual weights reached approximately one gram. At these times, static trials were begun and continued as each group reached weight increments of 5, 10, 20, 40, and 80 grams. Some species reached 20 grams, but because of loss of the original stocks of fishes, none of the tests was completed.

Harry D. Kennedy

ACUTE TOXICITY OF PESTICIDES TO FRESHWATER INVERTEBRATES

We determined the median tolerance limits (TL_{50}) of 16 herbicides by static bioassay to scud, *Gammarus fasciatus*, glass shrimp, *Palaemonetes kadiakensis*, crayfish, *Orconectes nais*, seed shrimp, *Cypridoposis vidua*, water fleas, *Daphnia magna*, and sowbugs, *Asellus brevicaudus*. Dichlone was the most toxic herbicide tested against the six species of crustacea with 48-hour TL_{50} values of 0.025 mg/l for water fleas to 3.2 mg/l for crayfish. The propylene glycol butyl ether esters of 2,4-D and silvex are more toxic to the crustacea than is the butoxyethanol ester.

Static bioassays with naiads of damselflies, *Ischnura verticalis*, and burrowing mayflies, *Hexagenia bilineata*, and a midge larvae, *Tanytarsus diisimilus* provided TL_{50} values for 25 insecticides. Toxicities of the insecticides to damselflies ranged from highly toxic methyl parathion (96-hour TL_{50} value of 0.00064 mg/l) to much less toxic Zectran (96-hour TL_{50} value of 0.1 mg/l). The most toxic insecticide to mayflies was carbophenothion (96-hour TL_{50} value of 0.006 mg/l), and the least toxic was malathion (96-hour TL_{50} value of 0.32 mg/l). Dursban and malathion are more toxic to midge larvae than DDT or aldrin.

Table 1.--Acute toxic interaction of pesticide combinations to rainbow trout and bluegills.

Pesticide combination Compound A	Compound B	Toxic interaction
DDT	Vapona	Additive
"	Endrin	Additive
"	Dieldrin	Additive
"	Azinphosmethyl	Additive
"	Toxaphene	Additive[1/]
"	Zectran	Additive
"	BHC	Synergistic[1/]
Parathion	Copper sulfate	Synergistic
"	Diazinon	Synergistic
"	DDT	Additive[1/]
"	Endosulfan	Additive
"	Methoxychlor	Synergistic [1/]
Malathion	Baytex	Synergistic
"	Copper sulfate	Antagonistic
"	DDT	Additive
"	EPN	Synergistic
"	Parathion	Synergistic
"	Perthane	Synergistic[1/]
"	Carbaryl	Synergistic[1/]
"	Toxaphene	Additive[1/]
Carbaryl	Copper sulfate	Synergistic
"	DDT	Additive
"	Azinphosmethyl	Additive[1/]
"	Methoxychlor	Additive
"	Parathion	Additive[1/]
Methyl parathion	DDT	Additive
"	Endosulfan	Additive[1/]
"	Carbaryl	Additive[1/]
Bidrin	Sumithion	Additive

[1/] This combination recommended for control of insect pests by the U. S. Department of Agriculture.

Note: Mention of trade names does not constitute endorsement.

We conducted continuous-flow bioassays for 30 days to determine extended-term toxicities of DDT, endrin, and malathion to crayfish. The TL_{50} values for the three insecticides decreased rapidly with time of exposure for the first 5 days, but decreased less rapidly for the subsequent 10 days. However, no further decrease in TL_{50} values occurred during an additional 15 days of exposure.

We exposed late instar burrowing mayfly naiads to malathion for 20 days in a continuous-flow bioassay. The toxicity of malathion increased with time of exposure from 0.55 mg/l after 24 hours to 0.15 mg/l after 5 days. This was followed by a less rapid decrease for the next 5 days (0.10 mg/l) and little change was recorded between 10 and 20 days.

The influence of selected pesticides on several species of invertebrates was measured in a Warburg respirometer. The study was designed to elucidate how pesticides exert lethal effects on fish-food organisms. Water fleas exposed to DDT and methoxychlor showed an immediate increase in oxygen uptake followed by a gradual decline until death. However, with chlordan we observed a 200-min. latent period followed by an abrupt rise in respiration and then a sharp decline in oxygen uptake. In general, the rates of oxygen uptake were influenced by variables such as toxicant concentration, activity of the animals, body size, and stage in their life cycle.

We shipped stocks of water fleas, seed shrimp, scud and glass shrimp to the Department of Forestry, United Kingdom, in the Azores to be used in populating a sterile lake, Lake Furnas, with fish-food organisms. Fortunately, we were able to work out proper methods for holding and packaging the animals for the long air shipment. We were most concerned with temperature changes, accumulation of metabolic wastes, and other conditions relating to organism density and water volume. The organisms arrived in good health. We were pleased to be able to cooperate with the Division of Fishery Services, Department of Forestry, U. K., and the U. S. Air Force in this matter.

Herman O. Sanders

CHRONIC TOXICITY OF INSECTICIDES TO COLD-WATER FISH

A study of the long-term effects of methoxychlor in bath and in the diets of cutthroat trout was completed at the NFH, Jackson, Wyo., during 1969. We found a significant relation between the concentration of methoxychlor and mortality and growth of adults, and the ripening of females. In a series of bioassays the progeny from fish in the high concentration bath treatment were more resistant to methoxychlor than progeny from the controls. The latter portion of the experiment was terminated prematurely when a bacterial epidemic necessitated removing all fish from the hatchery. The entire hatchery complex was disinfected on June 25, 1969.

The hatchery disinfection changed our experimental designs to use those fish available at Jackson for the next year. The wet laboratory was converted from an egg-incubating, fry-holding facility to a temperature controlled, 22-jar static bioassay facility. In addition, a battery of eight, 170-liter aluminum tanks is being constructed for short-term feeding experiments. The bioassay facility is operational and is being used to determine the relative acute toxicities of a variety of compounds to juvenile cutthroat trout (Table 2).

Dieldrin was approximately 1.75 times more toxic to juvenile cutthroat trout than photodieldrin. Many of the PCBs seem to have had a delayed effect on trout because most of the deaths occurred after 50-60 hours of exposure.

Our modified facility at Jackson is programmed for feeding trials during 1970. We have initiated two studies, one with chlordane and the other with PCBs, to measure tissue residues of these chemicals and their effects on serum alpha amino nitrogen, thyroid activity, growth, and mortality.

Don Swedburg

Table 2.--Toxicity of 12 chemicals to cutthroat trout at 8 C.

Chemical	TL_{50} values and 95 percent confidence limits in μg/l after-- 24 hrs.		48 hrs.		96 hrs.	
Antimycin A	0.49	(0.28- 0.64)	0.20	(.09- .30)	0.11	(.06- .16)
DDT	11.7	(9.2 -14.1)	9.7	(8.3 -11.0)	9.4	(7.6 -11.1)
Dieldrin	12.7	(11.8 -13.6)	8.5	(7.0 -10.0)	6.4	(5.3 - 7.5)
Photodieldrin	19.6	(17.9 -21.2)	14.0	(12.6 -15.3)	11.1	(9.8 -12.3)
Methoxychlor	19.3	(13.5 -25.0)	14.2	(10.06-17.7)	13.8	(12.4 -15.1)
Aroclor 1242[1/]					4600 - 8200	
Aroclor 1232	5900 - 10,3000		2600 - 4200		1900 - 2500	
Aroclor 1248					25,000	
Aroclor 1254					25,000	
Aroclor 5460					25,000	
Aroclor 4465					25,000	
Aroclor 5442					25,000	
Aroclor 1260					25,000	

1/ Preliminary data on Aroclor compounds.

PESTICIDES AND AQUATIC ANIMALS IN THE ESTUARINE AND MARINE ENVIRONMENT

Acquisition of suitable bioassay animals throughout the year continues to be one of our biggest problems. However, we are able to collect shiner perch, English sole, and speckled sanddab on a fairly regular basis. The Korean shrimp proved to be an excellent test animal because it is hardy and easy to hold in the laboratory. Its range appears to be diminishing, reportedly because of pesticides. We observed these shrimp were susceptible to what were normally sub-lethal concentrations of DDT. Long-term exposures of shrimp to low concentrations of DDT suggest a correlation between susceptibility and molting. In one 38-day trial with 20 individuals, 8 out of 10 dead shrimp were molting or had just molted, whereas none of the controls died.

Turbidity and salinity fluctuate widely throughout the year in San Francisco Bay. Even after filtration we have measured turbidities as high as 40 Jackson Turbidity Units in our laboratory water. Therefore, we have tested 10 organochlorine insecticides for their relative sorption on different amounts of sediments at three salinities. As expected, pesticide sorption increased with turbidity, however, we also found that sorption on sediment increased at higher salinities. Endrin had the least affinity for Bay sediments and aldrin had the highest.

Sediments in our laboratory water bear various amounts of DDT and the DDT complex. Because marine fishes drink water, we postulated that DDT residues in our experimental fishes may be related to the degree of turbidity. We initiated preliminary research which involved maintaining seven species of fish at three levels of turbidity. A trend toward higher DDT residues in individuals was indicated in these fish maintained at higher turbidity.

Russell Earnest and Pete Benville

CHRONIC TOXICITY OF PESTICIDES TO FISH

We extended our study of chronic effects of pesticides and their fate in fish to include carbaryl and channel catfish. Four duplicate groups (eight) of fish were exposed to ^{14}C-carbaryl in 500-liter tanks, two by bath and two by diet for 56 days. The insecticide was metered continuously for the bath treatments to give concentrations of 0.05 or 0.25 mg/l, or fed at the rate of 0.4 or 0.04 mg of labeled carbaryl/kg of body weight daily.

Table 3.--Residues of radioactive materials (mg/kg body weight) accumulated by channel catfish exposed by bath or in diet ($\bar{X} \pm$ SE, N=10)

Treatment	Exposure 3 days	8 days	14 days	28 days	56 days
Low-carbaryl bath (0.05 mg/l)	T[1/]	1	1	2	2
High-carbaryl bath (0.25 mg/l)	3 ± 0.3	7 ± 0.5	8 ± 0.5	9 ± 0.6	11 ± 0.4
Low-carbaryl diet[2/] (0.04 mg/kg per day)	2	T	T	T	1
High-carbaryl diet[2/] (0.4 mg/kg per day)	22 ± 2.6	6 ± 0.4	7 ± 0.7	7 ± 0.8	9 ± 0.3

1/ Trace, less than 1 ng/g.

2/ The fish were fed 5 hr before the 3-day sampling and 24 hr before the 8-, 14-, 28-, and 56-day samplings. The stomachs of all fish were cleaned at sampling.

The uptake, metabolite composition, and elimination half-life of radioactive carbaryl residues were determined by liquid scintillation counting (Table 3).

Fish exposed to the high diet concentration of carbaryl retained 89 percent of their radioactive residues after 25 days on control foods, whereas those exposed to the high bath treatment retained only 18 ± 3 percent after the same interval.

Carbaryl appeared to be excreted or metabolized at a rapid rate. Presently, we are trying to elucidate the composition of the radioactive metabolites.

Sidney Korn

PESTICIDE-INDUCED CHANGES IN POND ECOLOGY

Mosquito control agencies recommend Dursban to control the larval stages. But, our static bioassays indicate an adverse effect on fish populations if recommended application rates are used on ponds. Therefore, we studied the chronic effects of multiple treatments of Dursban on fish and fish-food organisms.

We applied 0.01 or 0.05 lb. of Dursban/acre to duplicate ponds and two additional ponds served as controls. Dursban was applied twice, June 9 and July 14. The mortality of bluegills and largemouth bass was 46 and 55 percent, respectively. Dursban residues in bluegills peaked seven days after the first treatment and three days after the second treatment. However, the maximal concentration of residues after the second treatment was twice the maximal residue concentration after the first.

Dursban residues in largemouth bass did not follow the bluegill pattern. Both peaks occurred three days after application, but the magnitude of the first treatment was twice that of the second.

Dursban changed population density and structure of non-target invertebrates. A density-time plot of bottom fauna in the control ponds had a cyclic pattern similar to the pattern for emergent forms. The sequence of increases and decreases in numbers of individuals in the ponds treated with 0.01 and 0.05 lb/acre was not similar to that of the control ponds. Estimates of changes in population structure and density in ponds treated at 0.01 lb/acre were intermediate between control ponds and those heavily treated. Therefore, the effects of Dursban appear to be correlated with treatment levels.

We could not demonstrate conclusive evidence of pesticide-induced pathology in our microscopial examination of Dursban-exposed fish. However, in ponds treated with 0.05 lb/acre, we found a moderate incidence of chronic splenomegaly after 28 days of exposure. Both species of fish were heavily parasitized at the start of the experiment. Mechanical damage to the liver, spleen, pancreas, kidney, gonad, lobulation of liver parenchyma and eosinophilic leucocytosis near or in the abdominal cavity characterized tissue responses to trematodes in the largemouth bass. The parasite damage to tissues and organs may have masked damage attributable to Dursban. The number and incidence of infestation of trichodina, trematodes, and mxyospordia in the gills of bluegills declined sharply in the 28-day samples.

Obviously, recommended application rates of Dursban for mosquito control are hazardous to fish and fish-food organisms. We are now better able to predict what effects this insecticide will have on pond ecosystems.

Similar to Dursban, our earlier laboratory bioassays suggested that a more intensive investigation was needed to predict potential hazards from the promiscuous use of parathion and malathion. These trials were conducted in ponds with green sunfish and channel catfish. Nine quarter-acre ponds were treated with the following concentrations: three ponds - 0.5 lb. of malathion/acre; three ponds - 0.5 lb. of parathion/acre; and three ponds - 0.5 lb/acre of each pesticide. Two additional ponds served as controls. The ponds were treated twice at 30-day intervals. After the second application nearly all the fish were killed in ponds treated with the pesticide mixture, but there were few deaths in ponds treated with only one insecticide. Samples of water, mud, and fish were collected for residue analysis, but the analyses are not completed. The invertebrates from ponds treated with the insecticide combination were unable to repopulate the ponds before the second treatment. In contrast, invertebrate populations in ponds treated with only one insecticide declined immediately after the application, but approached densities of the control ponds before the second application.

We determined that malathion and parathion acted synergistically (see acute toxicity section) in static bioassays, and the pond applications appear to support this finding. The final report will soon be completed for publication.

Thomas Russell of the Missouri Department of Conservation artifically spawned paddlefish in one of our tenth-acre ponds. Because of the surface swimming habits of very young paddlefish (Figure 1) and heavy predation by a pair of green herons and kingfishers, less than 50 survived. The remaining fish were moved to a private pond with a greater mean depth for overwintering.

David F. Walsh

INTERACTION BETWEEN MICROORGANISMS AND PESTICIDES

Our efforts to determine the effects of pesticides on the biotic communities of an aquatic ecosystem involved four specific areas of study: the influence of pesticides on primary producers; the bioconcentration and biopassage of pesticides; the influence of pesticide residues on individual trophic levels of a food chain; and the degradation of pesticides by members of the biotic community.

In a collaborative study with Dr. Robert Campbell and Mr. Lelyn Stadnyk, Department of Zoology, University of Missouri, we developed a suitable method for studying the effects of

Figure 1.--Immature paddlefish reared in a tenth-acre pond at the Fish-Pesticide Research Laboratory.

pesticide interaction on primary production in an aquatic environment. A manuscript entitled "The effect of selected pesticides upon growth and carbon uptake of Scenedesmus quadricauda" is near completion.

Herman Sanders and I are currently developing simple combinations of aquatic food chains designed to investigate biopassage and bioconcentration of pesticides under controlled laboratory conditions. A food-chain sequence of algae-daphnia-bluegill-bass shows the most promise. The investigation of uptake, retention and metabolism of ^{14}C-labeled DDT by freshwater invertebrates was completed. The crustacea Daphnia magna (waterflea), Gammarus fasciatus (scud), and Palaemonetes kadikensis (glass shrimp), and the immature aquatic insects Ischnura (damselfly), Hexagenia (mayfly) and Odonata (dragonfly) were exposed to 100 ng/l ^{14}C-DDT in a continuous flow system. The invertebrates contained residues from 1,000 to 100,000 times greater than those in water. D. magna, for example, accumulated 10 mg of DDT per kg within 48 hours. Sites of ^{14}C-DDT accumulation in this animal are shown in Figure 2. Furthermore, D. magna that were washed, returned to fresh water and fed, retained approximately 50 percent of their original DDT residues after 7 days. The rapid bioconcentration and retention of DDT by D. magna demonstrates the significant role many invertebrates may play in the entrance of pesticides into an aquatic food chain--even from a seemingly insignificant amount of pollution.

In preliminary investigations heptachlor was accumulated rapidly by microorganisms suspended in water. In 72 hours, pure cultures of Alternaria, Aspergillus, Bacillus, Chaetomium, Kurthia, Mucor, Mycobacterium, Nocardia, Rhizopus and Trichoderma concentrated over 50 percent of the insecticide contained in a concentration of 1 mg/l. Similarly, we found that bacteria isolated from intestinal tracts of fish concentrate ^{14}C-labeled DDT from water. DDT is concentrated by a factor of 100 to 1,000 times in 24-hour cultures. We feel that retention or organochlorine insecticides by microorganisms is essentially a passive adsorption phenomenon.

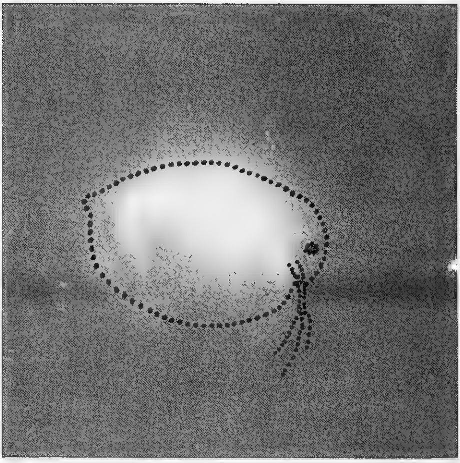


Figure 2.--Autoradiogram of D. magna exposed to ^{14}C-DDT.

We investigated the influence of heptachlor (1 mg/l) on growth (biomass) of selected microorganisms. The insecticide had marked effects on fungi, actinomycetes and gram positive bacteria. For instance, a 72-hour culture of Aspergillus niger increased in biomass by 14 percent while cultures of Kurthia zopfil decreased by 50 percent. Significantly, the gram-negative bacteria Aerobacter, Aeromonas, Achromobacter, Flavobacter, and Pseudomonas were not affected by heptachlor.

The degradation of DDT to DDD by bacteria isolated from the gastrointestinal tracts of channel catfish, bluegills, and largemouth bass was reported last year. In recent studies on degradation of ^{14}C-labeled DDT by gastrointestinal microflora of fish, supported by autoradiograms of thin-layer chromatograms, we found DDE, DDMU, DDMS, DBP, Kelthane, DDA, DBH, and several unidentifiable products in addition to DDD. Thus, a relatively broad spectrum of bacterial species can readily attack the ethane moiety of the DDT molecule under specific environmental conditions. The effects of these degradation products on the biotic community are still unknown.

I also participated with Dr. Charles O. Knowles, toxicologist at the University of Missouri, in a joint investigation involving the ability of selected microorganisms to degrade the acaricide, Galecron. Compounds containing an aniline moiety such as Galecron, Karsil, Propanil, and some phenylureas are converted under certain conditions to azo derivatives. The anilines, because of their potential carcinogenicity, are of considerable interest as environmental pollutants.

Our research on the metabolism of DDT by aquatic invertebrates (immature aquatic insects and crustacea) is complete. Basically, we found that DDT taken up by these organisms is changed within their tissues. Extensive metabolism of DDT to DDD, DDE, and Kelthane is indicated by all of our residue studies.

PESTICIDE EFFECTS ON FISH ENDOCRINE FUNCTION

We continued our efforts to elucidate possible mechanisms and effective levels of insecticide interference with growth and morphogenesis in fishes by studying thyroidal activities of pesticide-exposed rainbow trout. Two dietary doses of DDT or dieldrin, 0.145 mg and 0.029 mg/kg body weight per day and 0.145 mg/kg of both compounds in combination per day, suppressed thyroidal radioiodine uptake. Uptake was reduced 33.6 percent and 21.7 percent below controls by the high and low DDT dosage, 24.5 percent and 30.8 percent below controls by the high and low dieldrin dosage, and 15.4 percent below the controls by the DDT-dieldrin dosage, respectively.

We fractionated carp pituitary extract by gel filtration to isolate fish thyrotrophin for reinjection studies that will hopefully determine whether a toxicant acts directly on the thyroid gland or whether its effects are mediated through the pituitary. We replicated the preparative separation four times to attain the necessary amount of an active thyrotrophin principle for repeated injections in a future series of experiments.

The effects of a piscine adrenocorticotrophin (ACTH) on the circulating blood level of adrenocorticoids in goldfish are under study. ACTH-injected (crude pituitary extract) fish and controls were sampled serially at 2, 4, 6, and 8 hours after injection to determine the time of maximal response to a single ACTH injection. Serum cortisol peaked at 2 hours and declined linearly to twice the control's cortisol level at 8 hours. Then, in a separate experiment, we varied the dosage and sampled all groups at the 2-hour peak to disclose the response (serum cortisol level) to log dose (ACTH injected) relationship. Both experiments were remarkably successful and represent a major breakthrough in characterizing the stress response in fishes. In future experimentation using rainbow trout and channel catfish, we shall observe the induced response in the presence of selected pesticides to test for interrenal (adrenal) dysfunction. Using the blood cortisol response as a bioassay, we can follow the activity in isolating piscine ACTH by gel filtration.

We continued research into the effects of pesticides and two organic solvents on the induced spermiation response in goldfish. Metering pumps and flow regulators were installed and proved acceptable for continuous-flow administration of toxicants (Figure 3). Adult, male goldfish with developed testes were injected with the spermiation gonadotrophin (isolated from carp pituitary extract by Sephadex G-100 gel filtration). This induced the 24-hour spermiation response after the fish had received 6-8 days of exposure to a toxicant or solvent. Individual groups of fish were exposed to the

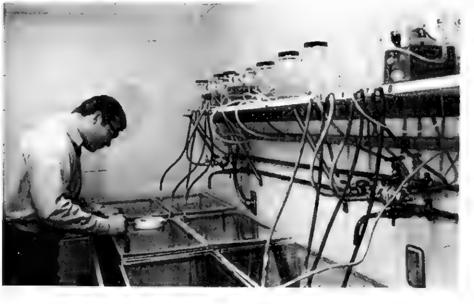


Figure 3.--Metering pumps and flow regulators for continuous administration of pesticides in physiological experiments.

dimethylamine salt of 2,4-D(700, 300, 150, 70 μg/l, and controls) and Dursban (4, 8, 18, 40 μg/l, and controls). The spermiation response occurred in all 2,4-D-treated groups, but the magnitude of response was proportionately less in the three highest-treatment groups and unaffected in the lowest-treatment group. The recommended treatment of 2 to 4 lb. of 2,4-D per acre (0.7 to 1.5 mg/l) may interfere directly with spawning in goldfish and could be more detrimental to less hardy species. On the other hand, Dursban did not affect spermiation even though brain acetylcholinesterase in all treatment groups was inhibited more than 90 percent. Classic symptoms of cholinesterase inhibition occurred in fish at all levels of treatment: torpor, and protraction of fins, and mouth. This demonstrated decidedly that induced spermiation was independent of central nervous system control.

The recommended application rate of Abate for mosquito control, 40 μg/l, did not affect spermiation nor brain acetylcholinesterase. Ethanol or acetone in concentration of 0.067 and 0.05 percent, respectively, also had no effect on spermiation nor on brain acetylcholinesterase. We attempted to administer simazine in bath exposure, but we were unsuccessful because of the compound's extremely low water solubility. However, by flowing the bath water slowly through a millipore filter containing simazine crystals, we hoped to achieve a saturated solution, but this solution had no effect on spermiation. Even toxic concentrations of chlordan did not affect induced spermiation. Thus, all of the compounds tested to date except 2,4-D appear to have no effect on induced spermiation. But, to successfully induce spermiation, fish must have undergone successful gametogenesis and we doubt that the gametogenic response is similarly refractory to the variety of toxicants we have used on spermiation.

In addition to the above bath exposures, we also tested injections of DDT and malathion against induced spermiation in goldfish. After massive, single doses of malathion (0.1, 0.3, and 1.0 mg/gm body weight), the fish immediately showed considerable distress such as fin and mouth distention, lethargy, and slow return of sigmoid body flexures to normal. By the following morning, the symptoms had disappeared and the spermiation response was similar in the controls and the treated groups. Similarly, single injections of 0.1, 0.3, and 1.0 mg DDT/gm body weight in quota of 10.0 μl/gm body weight, were given to mature male goldfish. These doses were higher than an intended sublethal range. Twenty-four hours after injection, the 0.3 and 1.0 mg/gm groups of seven fish had one and two deaths, respectively, and the spermiation assays were not continued. However, blood was collected from the surviving fish to determine whether death was associated with osmoregulatory failure. For this assessment, we analyzed the serum Na, K, and blood concentration of DDT. The sodium values for the 0.1, 0.3, and 1.0 mg/gm groups were 143.7, 128.9, and 127.9 milliequivalent/liter (mEq/l), respectively. Control goldfish had 141.5 $\pm$ 3.4 mEq/l (SD, N=14). The potassium values were all high: 5.0, 6.2, and 5.3 mEq/l respectively. The corresponding concentrations of DDT in the serum were 2.5, 7.3, and 11.9 mg/l respectively. The depression of serum sodium correlated statistically with the concentrations of DDT.

Immature rainbow trout were tested to determine whether ovine prolactin (mammalian homologue of piscine paralactin) exerts a diurnal lipogenic effect as previous work has shown it to do in sexually mature plains killifish. Apparently, a diurnal lipogenic response does not exist in immature rainbow trout. Part of this study consisted of subjecting the prolactin-injected and the control groups to 0.7 μg/l of endrin by continuous-flow exposure. The control fish all died within 48 hours, but the prolactin-injected fish lived for over 7 days.

In previous studies serum amino acid, creatinine, and non-protein nitrogen levels in fish appeared to be influenced by exposures to several insecticides. Therefore, we considered the amino acids to be of compelling interest and importance in elucidating mechanisms of likely pesticide-induced dysfunctions in nitrogen metabolism. We exposed rainbow trout to 1.0 mg of DDT/kg body weight or 1.0 mg of dieldrin/kg body weight by diet for 140 days, then subjected them to forced-swimming for 24 hr at 2 ft/sec.

Amino acids in 50μl of serum were quantitated by gas-liquid chromatography. Total amino acid concentrations were similar in the control, DDT, and dieldrin-treated fish, but several individual amino acids varied significantly. DDT caused serine, methionine, phenylalnine, aspartate, glutamate, and tyrosine to increase, whereas lysine, valine, trypthophan and cystine decreased. Dieldrin caused alanine, glycine, isoleucine, proline, threonine, phenylalanine, aspartate, and glutamate to increase, whereas lysine, histidine, tryptophan, and cystine decreased. After 24 hours of forced-swimming, total amino acid concentrations in the control and DDT-treated fish decreased significantly, but total serum amino acids of fish exposed to dieldrin did not decrease.

In addition to the differential analysis of the amino acids above, serum glucose, liver glycogen, serum amino acids, total body lipid, total body nitrogen, and pesticide residues were determined during continuous forced-swimming for four weeks. Utilization of liver glycogen was inhibited by dieldrin during the first week of forced swimming, but then the glycogen concentration decreased to the level of the control and DDT treated groups for the remaining period of forced swimming. After the first week of forced swimming, serum amino acids increased markedly in the dieldrin group, whereas those of the DDT group remained similar to amino acids of the control group. Lipid content was greater in the dieldrin-treated fish than in the DDT-treated and control groups during the first week of forced swimming, but total lipids in all groups decreased to a similar level for the remainder of the forced swimming period. Amino acids were apparently utilized preferentially for energy by dieldrin-treated fish during the first week of forced swimming, whereas DDT-exposed and control fish preferentially utilized fat and carbohydrate during this period.

We had an opportunity to collect blood from spawning paddlefish through cooperation with Thomas R. Russell of the Missouri Department of Conservation. Polyodon and related primitive groups of fishes have not been investigated to any appreciable depth, and we considered them of great general interest because of their unique phylogenetic position and because of their potential economic importance. The serum parameters characterized and summarized in a manuscript are: Na, K, Mg, Ca, Cl, inorganic phosphate, osmolality, cholesterol, NPN, total protein, protein electrophoresis, lactate, glucose, and cortisol.

Blake F. Grant and Paul M. Mehrle

METABOLISM OF PESTICIDES

During 1969, we examined the uptake kinetics and distribution of two insecticides and a herbicide. Rainbow trout were exposed to ^{14}C-DDT, ^{14}C-dieldrin or to both radioactive insecticides in combination. The chemicals were incorporated into their diets and fed at the rate of 1 mg/kg/wk over a period of 168 days. Residues of the pesticides occurred in all tissues samples including brain, liver, pyloric ceaca, megenteric fats, lateral-line muscle, striated muscle, gill and blood. The concentrations increased during exposure, but plateaued before the experiment was terminated. The highest residues were found in mesenteric fats and pyloric ceaca.

Individuals from all three of the above types of insecticide treatments were entered into two additional experiments. In one study, the fish were fasted, while in the other the fish were stressed by fasting and forced exercise. The exercise consisted of 4 weeks of forced swimming in a stamina tunnel at a velocity of 2 feet per second. Individuals were collected at intervals and analyzed to determine what effect the stress may have on weight and the distribution of DDT and dieldrin within tissues. Stressed dieldrin-treated fish lost 32 percent more weight during the first two weeks of stress than stressed-controls or stressed, DDT-treated fish. There were no significant differences between the relative weight losses of DDT-treated and control groups in either the stress or fasting experiments. However, stressed, DDT-treated fish and stressed controls lose 60 percent more weight than fasted groups of fish. The fasted groups of fish lost about the same amount of weight whether or not they were exposed to the insecticides.

The concentration of DDT in mesenteric fats of stressed, DDT-treated fish increased threefold during the four weeks of exercise, suggesting that DDT was not rapidly mobilized as fats were consumed. However, when mesenteric fats were nearly depleted, mobilization of DDT occurred and residues increased greatly in brains and livers. The fish then became immobile and died.

We observed that dieldrin-treated fish which were fasted and exercised for two weeks eliminate some dieldrin from their mesenteric fat and other organs. Although these fish lost considerable weight, little fat, if any, was utilized during this period. Forced exercise of dieldrin-treated fish did not accelerate the elimination of the pesticide. Elimination proceeded at a rate predicted by the elimination half-life previously determined to be 40 ± 4 days. However, the rate of dieldrin elimination from stressed fish exposed to combinations of DDT and dieldrin was not what we might predict. The presence of DDT in the combination treatment appeared to block the elimination of dieldrin resulting in larger dieldrin residues. In contrast, dieldrin did not cause an increase in DDT residues, but it did slow the elimination of DDT.

The potential of the herbicide 2,4-D to accumulate in fish tissues was investigated by exposing bluegills to a radioactive formulation of the chemical in water, and in their diets. The bath treatment consisted of placing the fish in 2 mg/l of ^{14}C-labeled 2,4-D dimethylamine salt (2,4-D DMA) for up to four weeks. During this exposure, we observed a gradual increase in whole body residues, and in residues within individual organs. Individuals exposed for 28 days contained up to 0.69 μg/g of radioactive material. The gall bladder bile contained as much as 55.5 μg/g and we measured 0.20 μg/g in muscle. Bluegills fed a diet containing 2 mg/kg/wk of ^{14}C-labeled 2,4-D DMA for 3 weeks retained whole body residues of only 0.005 μg/g of radioactive material. Thus, uptake of 2,4-D DMA from water appears more important as a source of 2,4-D residues in fish than does uptake from their food.

Our paper entitled "Uptake, Distribution, and Elimination of Dietary ^{14}C-DDT in Rainbow Trout" has been submitted to the Journal of the Fisheries Research Board of Canada. One paper entitled "Uptake and Elimination of Simazine by Green Sunfish (Lepomis cyanellus)," and another, "2,4-D Butoxyethanol Ester Uptake, Distribution, and Elimination by Organs of Rainbow Trout, Channel Catfish, and Bluegill," were prepared for the Weed Science Society of America.

Charles Rodgers and David Stalling

INTERACTIONS BETWEEN PESTICIDES AND FISH ENZYME SYSTEMS

The inhibition of brain acetylcholinesterase (AChE) is used frequently in research and monitoring to evaluate the effect of certain pesticides on fish and other aquatic animals. We find a controversy concerning the influence of different variables on AChE activity, i.e., freezing and storage of samples, environmental temperatures, species, etc. Investigations of these variables were initiated in 1968 and continued in 1969.

We measured AChE activities in fish brains frozen in liquid nitrogen or at -20° C. and then stored at -80° C. and -20° C. for periods up to six months. Methods of freezing and length of storage do not have a statistically significant effect on the mean percent inhibition of brain AChE activity by an organophosphate pesticide. However, brains of control and pesticide-treated fish should be stored under similar conditions. Although freezing and storage have little effect on percent of AChE inhibition, these preservative techniques do influence specific activity of the enzyme. We were able to correlate length of storage with increased specific activity. We concluded that under controlled laboratory conditions where percent inhibition rather than specific activity is under study, freezing and storage of fish brains are acceptable procedures.

Sex and water temperature were investigated as potential sources of variation in AChE activity of bluegill brains. Ninty-three female and 141 male bluegills were collected from our ponds at monthly intervals beginning in May, 1968, and terminating in July, 1969. Their brain AChE activities were estimated and compared statistically according to seasonal water and air temperatures and sex. A significant exponential increase in AChE activity was measured with warmer water and air temperatures, but was not correlated with sex.

Daphnia magna and the crayfish Orconectes nais were tested for their exterase activities, and central nerve cords of the latter had relatively high activities. Thus, we measured the inhibition of this enzyme in crayfish by selected organophosphate pesticides in vitro. Crayfish esterase activity is approximately as susceptible to organophosphate poisoning as is brain AChE in channel catfish and bluegills. These trials were expanded to determine the relative in vitro potencies of seven anticholinesterase agents on esterases of damselfly naiads, crayfish, channel catfish, and bluegills. We discovered that the esterase activity of damselfly naiads is the most susceptible. For example, the inhibition of AChE potency by 50 percent (pI_{50}) for 2,2-dichlorovinyldimethyl phosphate (DDVP, dichlorvos, or Vapona) was 7.4, 6.3, 6.1, and 5.6 for damselfly naiads, channel catfish, crayfish, and bluegills, respectively.

Esterases in fish blood may offer some advantages over brain esterases in determining the effects of cholinesterase inhibitors. Because blood samples could be drawn before and after pesticide treatments, each fish could serve as its own control. We estimated esterase activities in the plasma of 14 species of fish representing 10 families. Plasma from chain pickerel and channel catfish had the highest rates of activity, based on the number of micromoles of acetylcholine hydrolyzed per ml of plasma per hour. We selected channel catfish for further studies of enzymatic properties of their plasma esterases. The optimum substrates and substrate concentrations were determined by using various choline and non-choline esters. Also, we measured the enzymatic activity of the enzyme in the presence of six cations and a number of selected anticholinesterase agents. Magnesium and manganese increase enzyme activity while calcium, copper, and nickel reduce it. Cobalt has no measurable effect. When we compared pI_{50} values obtained for various organophosphate pesticides with channel catfish brain and plasma esterases, the latter esterase appears to be slightly more resistant than the former. For instance, DFP, DDVP, and malathion gave pI_{50} values of 5.9, 6.2, and 3.2, respectively, for plasma esterase while the corresponding values for brain esterase were 6.1, 6.3, and 3.5.

James Hogan

ACETYL CHOLINESTERASE INHIBITION AND STAMINA IN SALMONIDS

Coho salmon and rainbow trout were exposed to malathion and subjected to forced swimming to determine the correlation between AChE inhibition and stamina. We found that 200 g/l of malathion inhibited brain AChE by 86 percent in coho salmon and their physical activity index was reduced by 50 percent. In rainbow trout, 175 μg/l of malathion reduced AChE activity by 68 percent while the activity index is depressed by 65 percent.

George Post
Colorado State University,
Contractor

PESTICIDE INDUCED MINERAL IMBALANCE IN FISH

Comments of both inside and outside reviewers on the tentative manuscript entitled, "Polyvalent mineral imbalance in organs and organ systems of cutthroat trout induced by intermittent chronic exposures to endrin," are in the process of being reconciled and resolved.

A segment of the above report has been prepared under the title of "Significant prolongation of coagulation time of blood of cutthroat trout induced by intermittent chronic exposures to endrin." This report has now had its first internal review and is being revised in preparation for its second internal review.

Eugene T. Oborn

METHODOLOGY IN CHEMICAL ANALYSIS AND SAMPLING

We made considerable progress in developing a promising, easily automated technique for cleanup of fish extracts that may bring about appreciable savings in money and manpower. The method uses the gel permeation principle, but differs from typical adsorption chromatography because separations are based on molecular size rather than polarity. This eliminates the need for strong chemical or physical conditions, thereby reducing the possibility of compound degradation. Sample cleanup is accomplished thru the use of a gel permeation column. This method will be entirely operational when additional data concerning recovery efficiency are generated over the next few months.

Recently, we began to evaluate an analytical procedure which gives much greater recovery efficiencies than classical partition methods. This technique is based on liquid/liquid partitioning, but differs from classical partition extracts. One phase is contained in a long tube and the other phase is introduced as small droplets which travel thru the tube. Our work with this procedure is in the initial development phase.

An improved method of grinding fish samples was devised by Pete Benville, chemist at Tiburon. Initially, large samples of fish are chopped into pieces, and then blended with dry ice in a stainless steel blender cup. This mixture is stored overnight in a freezer to allow the dry ice to sublime and the resulting fish powder is extracted. We anticipate that use of this procedure will increase the efficiency of our analytical section. In addition, it led to trials of a new extraction procedure which uses 1:4 mixture of fish and anhydrous sodium sulfate. The mixture is placed in a chromatography column and the pesticides are then eluted with an appropriate solvent. Recoveries are generally comparable to, or better than, those obtained with previous techniques.

Partition values for different types of fish fat were determined with two solvent systems. This information, which is of value in method design, will be published soon.

Several procedures described in the literature for cleanup of pesticide samples were tested and found inadequate for analyses of fish tissues. Among the procedures evaluated were florisil chromatography, partitioning, channel-layer chromatography, several versions of forced volatilization techniques (sweep-codistillation), and low temperature precipitation.

Our gas chromatograph-mass spectrometer (GC-MS) (Figure 4) was installed in August and we were successful in obtaining limited numbers of mass spectra of the more commonly used pesticides. GC-MS examination of several of the polychlorinated biphenyl (PCB) compounds revealed they were complex mixtures of biphenyls, substituted with 2, 3, 4, 5, or 6 chlorine atoms. Using GC-MS we were able to determine the presence of DDT in a synthetic mixture of PCB compounds.

Chlordan was also examined by GC-MS, but elucidation of the many isomers and compounds composing this material is not yet complete. This instrument has greatly increased our ability to study the effects of pesticides on fish and the breakdown of pesticides in aquatic ecosystems.

This year, greater emphasis was placed on the development of analytical methods for herbicide residues, because as yet, residue

Figure 4.--Gas chromatograph-mass spectrometer.

tolerances in fish have not been established by Food and Drug Administration. The benzyl ester derivative of dalapon was found to be suitable for GLC. However, while working with aqueous solutions of this herbicide, we discovered that it may degrade quite rapidly in water. For instance, 60 percent of 1,000 mg of ^{14}C-dalapon in 1 liter of water was hydrolyzed to pyruvic acid after standing 1 month at room temperature. Degradation is greatly reduced by storing dalapon solutions in the dark at 2 to 3° C.

We developed a method for analysis of 2,4-D dimethylamine salt (2,4-D DMA) in fish tissue which gives recoveries of 84 $\pm$ 3 percent with spiked samples. This method uses acidic methanol (H_3P0_4) extraction for cleanup; analysis is as the methyl ester. Green sunfish were exposed for 1 to 3 weeks to a 5 mg/l bath of ^{14}C-2,4-D DMA and analyzed. The free acid form of 2,4-D accounted for approximately 5 percent of the total activity. We do not know the identity of the remaining radioactive compounds, but the radioactivity is distributed between at least 2 compounds or groups of compounds. One of these compounds is relatively non-polar, having a polarity which is roughly comparable to the polarity of most organochlorine pesticides. The other compound, or group, is extremely polar, but apparently not charged. We think this group may contain different conjugates of 2,4-D.

Our work on the preparation of highly purified solvents culminated with the publication of a paper entitled "Purification Procedure for Low Polarity Solvents."

We have completed a manuscript entitled "A Handbook of Standard Methods of Analysis for Pesticide Residues." This manuscript will be published as the first issue of Fish-Pesticide Research, a proposed new series.

Roger Tindle, David Stalling,
Robert Hesselberg, and
Pete Benville

HISTOPATHOLOGY SECTION

Exposure of cutthroat trout (*Salmo clarki*) to endrin at the Jackson NFH caused lesions in their gills, livers, pancreas, and ovaries. Extravascular edema, hemorrhage, and possible intracapillary congestion causing globe-shaped lamellae characterized the gill damage noted in fish exposed to the higher concentration of endrin. Hepatic lesions in young trout were of the type frequently described as preceding the development of hepatomas in trout. The increased incidence and severity of liver changes observed in fish exposed to the greatest amount of endrin by bath and in food suggested a nutritional deficiency enhanced by exposures to endrin. In addition, these individuals had pronounced pancreatic islet hyperplasia and irregular, atypical oocytes.

John A. McCann, biologist, Agricultural Research Service, Pesticide Regulation Division, Beltsville, Md., alerted our laboratory to a macroscopic redness occurring in the caudal region of about 60 percent of 5 to 6 cm bluegills (*Lepomis macrochirus*) after exposure to 0.32 mg/l Dyrene or 4.9 mg/l Akton. We duplicated this experiment at Columbia and took photographs of a non-exposed control and a bluegill which was exposed to 0.32 mg/l Dyrene (Figure 5). In a preliminary microscopial examination of a transverse section of the hyperemic area, I found a partial recanalization of an occluded caudal artery, intermuscular hemorrhage, and degeneration of some muscle bundles.

Lafayette Eller

PESTICIDE ANALYSIS SECTION

Our section has made residue analyses requiring more than 3,000 pesticide determinations on 1,500 of our research samples. These analyses have provided necessary information to determine the effects of pesticide on fish. Considerable analytical support has been given to the chemical methodology section.

Our analytical capabilities were extended to at least 20 other government agencies. These agencies submitted 61 fish samples, 28 water samples, samples of two fish diets, and 4

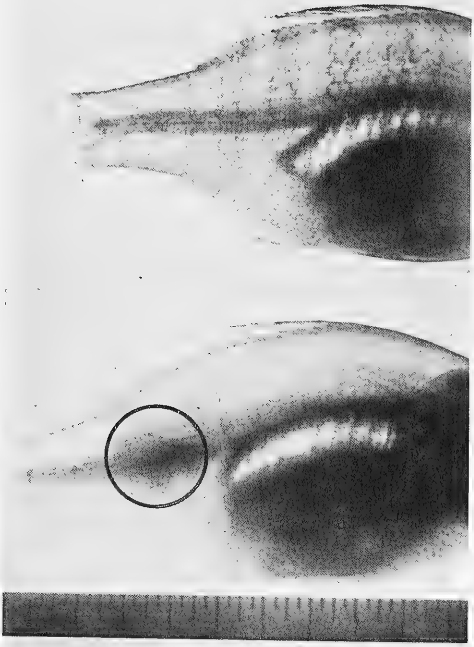


Figure 5.--Non-exposed bluegill (top) and a bluegill exposed to 0.32 mg Dyrene^R/1 for 17 hours (bottom). The encircled area on the lower photograph shows the grossly visible hyperemic area.

sediment samples. In addition, 10 National fish hatcheries submitted a variety of samples for pesticide analysis. Typical results include the determination that experimental fish diets were highly contaminated with dieldrin at the NFH, Spearfish, S. D., saving considerable development time and effort; the NFH, Craigbrook, Maine, conceivably saved a year of production time by anticipating high mortalities from potential pesticide residues in Atlantic salmon eggs. These analyses assist the agencies responsible for fish production and utilization.

Robert Hesselberg

FISH CULTURE SECTION

The Fish Culture Section was established as a separate unit in July, 1969. This section acquired 371,185 fish of 21 species in 1969, held 475,413 fish for research purposes, and assisted in planning projects using these fish. Incubation of 20,700 channel catfish and 58,172 rainbow trout eggs aided established work units. Over a ton of food was prepared throughout the year to hold these fish. The Division of Fish Hatcheries, Regions 3 and 4, the Southeastern Fish Cultural Laboratory, Marion, Ala., and the Fish-Farming Experimental Station, Stuttgart, Ark., gave us splendid cooperation in obtaining these valuable fish for research.

The appearance of gravid, female channel catfish in August provided me with the opportunity to spawn these fish much later than normal. Four pairs of two-year-old channel catfish spawned in late August and early November. Feeding high-protein diet, holding parent fish at low temperatures, and gradually increasing the temperature in the proper sequence were critical in bringing about spawning at this time. The induction of young fish to spawn took less time, money and space than spawning large brood stock in the IV to X age classes. Use of this technique will make gram size channel catfish available for mid-winter research and allow catfish farmers to delay channel catfish spawning until convenient.

Incubation of paddlefish eggs provided by the Missouri Department of Conservation at 17.2 C. for 5 days produced 500 sac fry. Some of the problems we encountered included egg sensitivity to strong light, pronounced adhesiveness of green eggs, and fungus infections. Malachite green treatments of the eggs for fungus at 17 mg/l for 10 min. has no effect on embryo development, but the fry did not accept artificial food and died.

Jim Brauhn

COOPERATIVE RESEARCH STUDIES

Determination of pesticide residues in fish from Lewis and Clark Reservoir assisted a study being conducted by the University of South Dakota on bald eagle populations.

We provided bioassay services for testing 38 compounds from 13 chemical companies, 3 compounds for the U. S. Coast Guard, Dyrene for U. S. D. A., and additional compounds from Fishery Services (Portland, Ore.); States of Wisconsin and Colorado; Stauffer Chemical Company; FWPCA (Alaska); American Cyanamid Company; and Radiant Electric Cooperative, Inc. (Kansas).

Dr. Stalling participated with Dr. Charles Gehrke, University of Missouri, in the Organic Analysis Consortium, NASA, Ames Research Center, in analyzing lunar samples from the Apollo 11 space flight. A new class of organic compounds, organosiloxanes, was discovered.

Herman Sanders cooperated with the Division of Fishery Services, USAF, and the Division of Forestry, U. K., in shipping stocks of fish-food crustacea to be used for populating Lake Furnas in the Azores.

Lafayette Eller contributed photomicrographs to Dr. Lawrence Roder, Argonne National Laboratories, Inc., to be included in a proposed text book, "Diseases of Laboratory Animals," Vol. I.

FISH CONTROL LABORATORIES

La Crosse, Wisconsin
Robert E. Lennon, Director

HIGHLIGHTS

Research at La Crosse was interrupted for the third time in five years by flooding of the Mississippi River, but there was no property damage.

Quinaldine sulfate, a water-soluble salt of quinaldine, is under development as a fish anesthetic. A synergic mixture of MS-222 with quinaldine sulfate is a highly effective anesthetic, having the quick knockdown rate of MS-222 and the extended holding time of quinaldine sulfate. Residues of quinaldine in anesthetized fish are reduced to background level in less than 24 hours after withdrawal.

Field trials in Florida indicate that walking catfish (Clarias sp.) are not repelled by antimycin, but the fish are as resistant to the toxicant as bullheads.

Bioassays in flowing water revealed that fish are killed by much shorter exposures to antimycin than to rotenone.

Continuing work on the biological activity of toxicants and anesthetics shows that the pH of the solution is critical in governing passage of a chemical through the gill into the fish.

The Laboratory at La Crosse hosted the Bureau's first Workshop for physiologists and biochemists.

We accepted a contract from FAO to do a review of toxicants in fish management.

INTRODUCTION

The Urban Renewal Authority of La Crosse and the Army Corps of Engineers have forced us to consider the future of the Laboratory. The principal reason for concern is the Harborview Plaza Urban Renewal Project that includes a portion of Riverside Park where we are located. Some of our basic utilities, such as municipal water, sewage, electricity, and telephone, pass through the Project Area. We face interruptions of utility service as well as limited capacities. The Project got underway in 1969, properties were purchased, some buildings were demolished, and accelerated activity is scheduled for early 1970. There is a possibility that public or commercial developments peripheral to the UR Project Area will impinge further on Laboratory property or operations.

The second major reason for concern is the Corps of Engineers' plan to deepen the Mississippi River channel from 9 to 12 feet soon, and to 15 or more feet later. The City may elect to use dredge spoil to raise the level of Riverside Park above flood stage or to erect a permanent levee to protect the Park. Either approach would leave the Laboratory in a hole, literally.

The City prefers we move the Laboratory to another site in La Crosse and will aid us in doing so. We prefer this approach because we need more laboratory space and experimental ponds to study chemical, biological, physical, and integrated controls for fish. Then, Wisconsin State University--La Crosse proposes that the Bureau and University cooperate to acquire a site and construct a fishery center, to include the Fish Control Laboratory, University facilities for teaching and research in aquatic biology, and a headquarters office for the Mississippi River Research Consortium. A public aquarium and visitor center may also be appropriate. City and State authorities have approved the proposal, and Congress appropriated a small sum for preliminary planning. The staff has assembled 129 pages of plans and drawings into "A Proposal for Expansion of the Fish Control Laboratory," and copies were submitted to Washington.

The third major flood in 5 years occurred on the Upper Mississippi in April. The Laboratory began sandbagging and other preventive activities on March 31; and arrangements were made with the City, Civil Defense, Corps of Engineers, and Coast Guard for mutual aid (Figure 1). Our experimental work ceased on April 14. The flood crested on April 20 below the predicted level, but the water lapped within one foot of the Laboratory building (Figures 2, 3, and 4). The flood receded rapidly which enabled us to start restoring the Lab to operable condition. Services and equipment were reinstalled, new supplies of fish obtained, and experiments resumed during the second week of May. There was no property damage, and the only loss was that of research time. This was, however, the third flood-related interruption of research, and it strengthens arguments for relocation of the Laboratory to another site in La Crosse.

We marked the fifth anniversary of research and efforts to get MS-222 registered as a fish anesthetic. The sponsoring company submitted a revised application for registration in July, but we have no word on its fate. The mechanics and time involved in registering fishery chemicals are discouraging.

Six students were employed part-time, including four majors in chemistry, one in biology, and one in education. Two faculty members were also employed part-time. We served as advisors on five research projects conducted by non-employee students.

We renewed a contract with the Biology Department, Black Hills State Teachers College, Spearfish, S. D., for a study on "The effects of

Figure 1.--Black River Falls, Wisconsin Boys Camp enrollees, filling sandbags for flood protection in the pool area at the Laboratory. Photo taken by Bernard L. Berger.

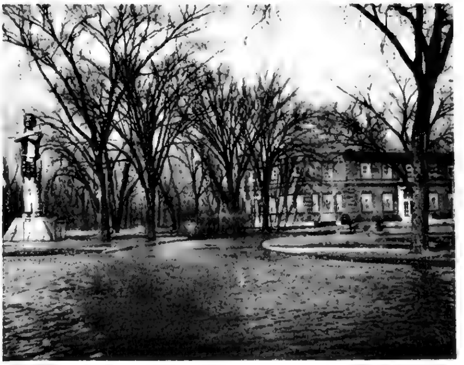


Figure 2.--Water level of 15.4' on the road southwest of the Laboratory on April 18. This was .4' below the flood crest of 15.8 which occurred on April 20. Photo taken by Bernard L. Berger.

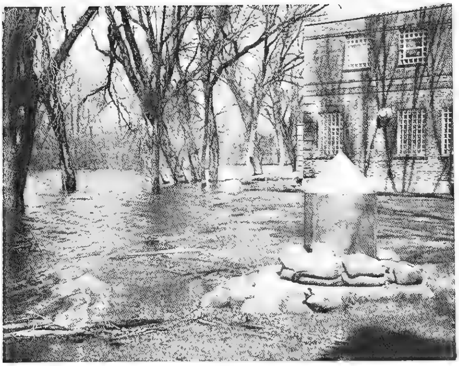


Figure 3.--Water level of 15.4' at the northwest corner of the Laboratory on April 18, .4' under the flood crest of 15.8' which occurred on April 20. Photo taken by Bernard L. Berger.

Figure 4.--Polyethylene and sandbags placed to protect loam and levee north of the Laboratory and Garage. Water level 15.4' on April 18, .4' below the April 20 crest of 15.8'. Photo taken by Bernard L. Berger.

magnetism on rainbow trout." We accepted a contract from the Food and Agriculture Organization of the United Nations to do a review of chemical means and methods used in fish control throughout the world in the past 30 years. This task is to be completed in the first half of 1970.

We provided advice and reviews on contract research by the Wisconsin Alumni Foundation for the Wisconsin Department of Natural Resources and by Wisconsin State University-La Crosse for the Max McGraw Wildlife Foundation. Antimycin played a large role in both studies (Figure 5).

Sixteen papers were published and 21 are in press. Seven special reports were completed. Fifteen major talks and many minor talks to tour groups and local organizations were presented.

Two staff members were appointed to faculty status at Wisconsin State University-La Crosse. We also hold appointments to committees in the National Academy of Sciences, Wisconsin Research Advisory Council, Wisconsin Scientific Areas Preservation Council, La Crosse County Civil Defense Office, and the American Fisheries Society. An appointment to the Interior Taconite Study Group was terminated. Twelve staff members participated in 19 training courses and 8 college courses.

Figure 5.--A registered fish management tool researched by the Fish Control Laboratories and now being widely used. Photo by Bernard L. Berger.

Two Reports of Invention were submitted to the Department's Solicitor.

There were 24 tours given to 1,306 people, 11 talks to 339 people at La Crosse and Warm Springs. Two seminars were presented to 60 people by personnel of both Labs.

Robert E. Lennon

SECTION OF CHEMICAL BIOASSAY

Toxicity of potential control chemicals to fish

Preliminary static bioassays included experimental fungicides, bactericides, avicides, insecticides, chemotherapeutants, chemosterility compounds, anesthetics, herbicides and a few reagent chemicals not designated for any specific use. Their activity ranged from lethal at 10 ppb to nontoxic at 100 ppm. Many of the chemicals show potential as fish toxicants. Some show species specificity while others are extremely toxic to all species of fish. The potential preliminary candidates were selected for delineation under controlled test conditions of water hardness, temperature, and pH.

Quinaldine sulfate ($QdSO_4$)

Quinaldine is a common fish anesthetic. Its advantages include economy and safety to fish under long exposure. Its disadvantages are insolubility in water, disagreeable odor, slow anesthesia, and liquid form. Chemists at the Warm Springs Laboratory improved the anesthetic by treating quinaldine with sulfuric acid. The result is quinaldine sulfate, a water-soluble powder easier to use and with far less disagreeable odor. A product description and structure identification have been prepared at Warm Springs. The data include UV, infrared, and mass spectra of the compound as well as the test for sulfates, elemental analysis, melting point, and purity as determined by acid equivalents. Additional work on the toxicity, efficacy, and residues of quinaldine sulfate is underway at La Crosse and Warm Springs to register the compound.

Quinaldine sulfate was tested against fish to establish toxic concentrations. Because previous testing at the Laboratory showed quinaldine is less toxic and less efficacious in acid pH water, we investigated the fate of quinaldine sulfate in different water qualities. The pH in very soft water drops from 6.55 to 3.86 with 80 ppm of quinaldine sulfate. In harder waters the pH is more stable and drops only 1 pH unit in very hard water (Table 1). Extremely high concentrations are necessary to kill rainbow trout in very soft water (Table 2). The 1-hr. LC_{50} was not determined because no fish died at 140 ppm, the highest concentration tested. At this concentration, the pH was 3.35. Thus, the toxic effects are diminished in acid water. Temperature is not as critical as pH and salt content of the test water in altering activity. In 1-hr. exposures, $QdSO_4$ is more toxic to fish at 7 C. than at other temperatures, but in 96-hr. exposures, it is more toxic at 17 C.

Salicylanilides as fish toxicants

High pH in some natural waters limits the effectiveness of fish toxicants. We are attempting to develop new toxicants that can perform satisfactorily in water at pH 6.0 to 10.0. Some salicylanilides are biologically active in high, low, and neutral pH waters. In our recent bioassays, salicylanilides with phenyl- and tertiary butyl- substitutions in addition to hydroxy-, nitro-, halo-, and alkyl- groups were highly toxic, offering chemical stability in problem pH waters. I selected 6 salicylanilides whose toxicity and persistence is influenced by different pH conditions (Table 3). All showed potential as general toxicants in preliminary tests, but in further tests some did not perform acceptably at high or low pH's. Persistence of these chemicals at pH 6.0 to 10.0 was checked by aging a series of bioassay solutions for a week before adding fish. Another series of fresh reference solutions were bioassayed concurrently with the aged solutions using bluegills from the same source. The LC_{50} was calculated for each series and the deactivation index, a measure of inactivation, was derived by dividing the LC_{50} of the aged test by the LC_{50} of the reference test. Index values near 1.0 indicate little deactivation within 1 week, whereas larger numbers such as 10 to 15 indicate more rapid deactivation.

Table 1.--The influence of quinaldine sulfate on pH in soft and hard waters.

Quinaldine sulfate (ppm)	pH in various water qualities [1/]			
	Very soft	Soft	Hard	Very hard
0	6.55	7.10	7.78	8.00
5	6.29	6.92	7.57	7.82
20	5.65	6.61	7.30	7.55
40	5.40	6.22	7.00	7.39
60	4.02	6.04	6.87	7.19
80	3.86	5.67	6.61	7.01

1/ Total hardness as $CaCO_3$ ranges from 10 ppm in very soft water to 320 ppm in very hard water.

In Table 3, the three chemicals on the right (Nos. 43489, 44016, and 52790) are p-chloro-phenyl-substituted, and their toxicity to bluegills is extremely diminished at pH 6. Cpd. 43057 is toxic at all pH's but more so in acid and neutral waters. It is also persistent. Cpd. 42317 is less toxic than most of the other salicylanilides and is deactivated more rapidly at pH 9 and 10. Cpd. 51294 shows greatest potential as a general toxicant. Its LC_{50}'s range from 3.69 to 13.50 ppb and deactivation indexes range from 1.06 to 1.70 at pH's 6 to 10. This tertiary butyl-salicylanilide provides good killing power and persistence at all pH levels.

These bioassays in chemically buffered solutions demonstrate that closely related chemicals differ significantly in toxic activity and persistence--themical structural configurations are important to biological activity. The studies also demonstrate how potential toxicants can be eliminated in the laboratory before use in field trials.

Antimycin: Half-life of biological activity

Knowledge on the persistence and degradation of antimycin in water is incomplete, especially in high pH waters known to influence deactivation. Analytical methods do not detect very low concentrations of antimycin in water. However, we must know how long the antibiotic remains at toxic levels in water of different pH's. We have developed a method to estimate the half-life of biological activity by testing differentially sensitive fishes in aging bioassay solutions. More resistant fish are exposed to fresher solutions of antimycin while more sensitive species are exposed to aged solutions that have become inactivated to some extent. LC_{50}'s are determined for the fish in aged solutions and also in concurrent reference tests containing a known series of concentrations. The percent reduction in LC_{50}'s reflects the rate of inactivation. The percent concentration remaining in a bioassay at a selected exposure is found by subtracting the percent reduction from 100. The procedure is illustrated by the following mathematical relationship:

$$\text{Percent concentration remaining} = 100 - \frac{(C_1 - C_2)100}{C_1} \quad \text{where}$$

$C_1 = LC_{50}$ of aged solution

$C_2 = LC_{50}$ of reference solution

The half-life of biological activity is found by plotting the percent concentrations remaining against time on cyclic semi-logarithmic graph paper.

We have used this method to estimate half-life of antimycin in waters chemically buffered to maintain pH's of 6 to 10 (Figure 6). At pH 6, the half-life of biological activity is 310 hours, but at pH 9, 9.5 and 10 the half-lives are 8.7, 4.6, and 1.5 hours, respectively. Half-lives may be even more brief in certain natural waters

Table 2.--Toxicity of quinaldine sulfate to rainbow trout in different water hardnesses and temperatures

Water hardness	Temp. (C.)	LC50 and 95 percent confidence interval (in ppm) at				
		1 hour	3 hours	6 hours	24 hours	96 hours
soft	7	28.90 24.29-34.39	28.45 24.65-32.84	25.78 22.62-29.38	23.75 20.25-27.86	23.75 20.25-27.86
soft	12	46.10 41.35-51.40	41.12 37.97-44.53	40.00 36.14-44.27	37.02 32.45-42.24	31.75 28.65-35.19
soft	17	47.00 29.52-55.87	45.99 40.60-52.10	42.50 34.95-51.68	23.79 19.20-29.48	12.82 11.05-14.88
very soft	12	---	133.00 115.89-152.64	90.50 83.21-98.42	65.50 62.18-69.00	50.50 45.96-55.47
hard	12	30.00 27.32-32.93	29.60 28.30-30.96	29.60 28.30-30.96	23.50 21.29-25.94	22.90 21.87-24.87
very hard	12	28.90 26.03-32.09	28.20 24.84-32.01	28.20 24.84-32.01	25.00 21.92-28.51	23.00 20.08-26.34

Table 3.--Toxicity of salicylanilides to bluegills in fresh ana aged (1 week) bioassay solutions

pH	Solution	96-hr. LC50 (ppb) of					
		Cpd. 51294	Cpd. 42317	Cpd. 43057	Cpd. 43489	Cpd. 44016	Cpd. 52790
6.0	fresh	3.69	94.30	9.30	500.00	400.00	400.00
	aged	6.29	160.90	11.40	2130.00	1800.00	2000.00
	D.I.[1/]	1.70	1.70	1.22	4.26		
7.4	fresh	5.72	46.90	13.20	39.50	57.00	30.00
	aged	6.50	100.90	17.20	264.00	1800.00	30.00
	D.I.	1.13	2.15	1.30	6.68		
8.0	fresh	6.00	43.70	17.20	28.50	49.40	16.40
	aged	8.06	81.40	17.40	120.00	150.00	21.50
	D.I.	1.34	1.86	1.01	4.21	3.03	1.31
9.0	fresh	8.29	62.80	28.60	31.00	77.00	13.40
	aged	12.24	316.50	42.60	74.80	644.00	20.00
	D.I.	1.47	5.03	1.48	2.41	8.36	1.49
10.0	fresh	13.50	126.00	100.00	51.50	212.00	25.60
	aged	14.35	793.40	105.00	930.00	2000.00	27.50
	D.I.	1.06	6.29	1.05	18.10	9.43	1.07

1/ Deactivation index.

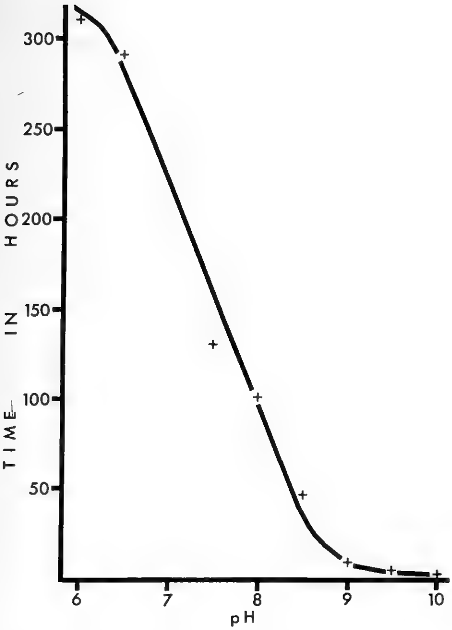


Figure 6.--The half-life of biological activity of antimycin at different pH's.

Antimycin inactivation

In previous tests antimycin appeared to be inactivated rapidly by high pH. They did not differentiate, however, between irreversible and reversible inactivation. In attempts to delineate the type of inactivation, we introduced bluegills at different times into bioassays of antimycin at pH 9. Concurrently, in another series, bluegills were introduced into bioassays in the same manner except that the solutions of antimycin were rebuffered from pH 9 to pH 7 just before adding fish. Figure 7 shows that antimycin is rapidly inactivated at pH 9, whereas its activity is restored almost completely when the solutions are rebuffered down to pH 7. Thus, the antimycin had not decomposed significantly, but the high pH water inhibited its killing power. Under conditions of fluctuating pH in natural waters, a presumed sublethal concentration of antimycin could become toxic to non-target organisms if the pH were to shift from basic to acid after the toxicant is applied.

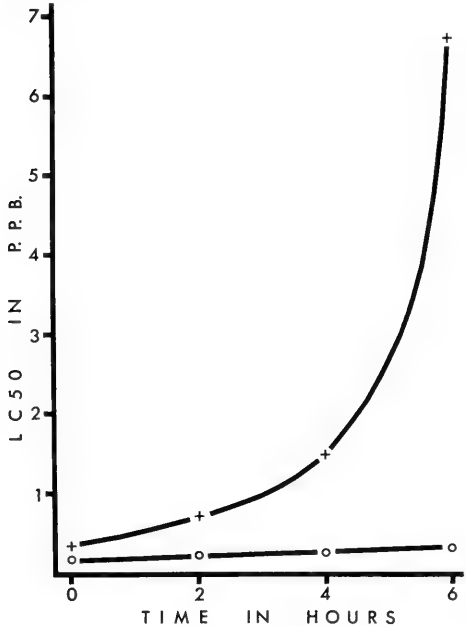


Figure 7.--The toxicity of antimycin to bluegills introduced to the bioassays from 0 to 6 hours following antimycin addition. Reference tests at pH 9 are represented by (+) and tests in water rebuffered from pH 9 to pH 7 are represented by (O).

In addition to pH, the ion content of the water has been reported to influence the inactivation of antimycin. Salts of $NaHCO_3$, $MgSO_4$, $CaSO_4$, and KCl were individually eliminated or increased by eight times their normal concentration in the test waters. The pH and alkalinity were monitored throughout the tests to more accurately assign the influential ions. The pH's were controlled with chemical buffers when necessary. Results in tests with rainbow trout and bluegills showed that deletions of $MgSO_4$, $CaSO_4$, and KCl had very little influence upon the toxicity of antimycin, or the pH and alkalinity of the solution (Table 4). By eliminating $NaHCO_3$, the antimycin became more toxic and the pH and

Table 4.--Toxicity of antimycin to fish in controlled pH solutions at 12 C.

Species	Changes in routine test water	pH	Total alkalinity as $CaCO_3$ (ppm)	96-hour LC_{50} (ppb)
Rainbow trout	None	7.30	30	0.048
" "	Buffered	7.10	88	0.038
" "	Eliminated $NaHCO_3$	5.50	2	0.027
" "	Eliminated $NaHCO_3$ and buffered 1/	7.10	8	0.040
" "	8 x $NaHCO_3$	8.18	220	0.083
" "	8 x $NaHCO_3$ and buffered 2/	7.11	256	0.040
Bluegill	None	7.30	30	0.220
"	Buffered	7.10	89	0.208
"	Eliminated $NaHCO_3$	5.30	1.2	0.057
"	Eliminated $NaHCO_3$ and buffered 2/	7.15	65	0.200
"	8 x $NaHCO_3$	8.15	220	0.468
"	8 x $NaHCO_3$ and buffered 2/	7.10	256	0.258

1/ TRIZMA(R) buffered

2/ Phosphate buffered

alkalinity dropped significantly. The reverse is true when $NaHCO_3$ concentrations are increased. However, when strong bicarbonate solutions were pH buffered to near original levels, they regained toxic activity against rainbow trout to routine test water. The LC_{50} of antimycin in water lacking $NaHCO_3$ is 0.027 ppb whereas that value is 0.040 ppb in buffered tests. The LC_{50} for rainbow trout in water with eight times the $NaHCO_3$ is 0.083 ppb, whereas it is 0.040 ppb again in buffered tests. Thus, $NaHCO_3$ influences the toxicity of antimycin against fish through pH manipulations only.

Fish therapeutants

Furpyrinol, 6-hydroxymethyl 2 [2-nitro-2-furyl) vinyl] pyridine, a potential bactericide from Japan was checked for its toxicity to fish. This compound is presently undergoing clearance for use in fisheries. The bactericide is not very toxic to trout, especially in short exposures (Table 5). Few fish died before 24 hours in saturated solutions (100 ppm) of the compound, but rainbow trout, carp, black bullheads and sunfish died within 72 hours. Also, compound is less toxic to rainbow trout in colder and harder water. The results of the toxicity tests are encouraging for eventual use of this therapeutant.

Juglone in different pH waters

Juglone, an extract of walnut, was tested against black bullheads in reconstituted waters at pH 6.0, 8.0, 8.5, 9.0, and 9.5 to determine its rate of inactivation. The bullheads were added to a series of newly prepared solutions of juglone and also to identical solutions which had aged one week. Juglone is effective against bullheads in the fresh solutions at about 100 ppb regardless of the pH. In the aged solutions, however, approximately double the amount of juglone was required to kill the fish at pH 9.0. At pH 9.5, bullheads were not completely eliminated at 1.0 ppm. The tests show that juglone is inactivated much faster at high pH than at low pH. The compound continues to show promise as an effective toxicant, especially in alkaline situations. No company is sponsoring this candidate toxicant, the cost for synthesized material is extremely high, and only research quantities are available or practical at the present time.

Table 5.--Toxicity of 10-percent Furpyrinol (P-7138) to rainbow trout in different water qualities and temperatures

Water hardness	Temp. (C.)	LC50 and 95-percent confidence interval (ppm) at 24 hours	48 hours	96 hours
soft	7	61.89 54.78-69.92	35.00 27.47-44.59	15.91 12.51-20.23
soft	12	50.40 47.45-54.58	44.00 40.78-47.48	15.89 14.04-17.98
soft	17	31.24 26.49-36.85	11.15 9.87-12.60	7.32 6.00- 8.93
very soft	12	1/	1/	7.40 5.61- 9.77
hard	12	46.60 40.63-53.44	15.89 13.05-19.34	8.13 6.89- 9.59
very hard	12	114.90 91.49-144.30	21.25 18.91-23.88	12.23 10.56-14.17

1/ Inconsistent mortality prevented data analysis.

Toxicity of herbicides to fish

Thirteen potential aquatic herbicides were tested against rainbow trout, goldfish, carp, black bullheads, channel catfish, green sunfish, bluegills and yellow perch. Most of the compounds are ethers of alkyl-substituted amines and are not particularly toxic to fish. One shows selective toxicity to carp. Several have been selected for further delineation. The sponsor is also working with the U. S. D. A. at Fort Lauderdale, Fla., and other research agencies in attempts to develop an aquatic herbicide safe in the presence of fish.

Toxicity of potential control chemicals to fish eggs

Six chemicals were tested against recently spawned rainbow trout eggs in reconstituted water at 12 C. Twenty-five eggs were exposed to each concentration in 2.5 liters of solution. Juglone is the most toxic of the materials tested and zinc sulfate is the least toxic (Table 6). In additional tests, juglone killed eggs equally well in very soft and very hard reconstituted water and also in water that was buffered to pH 9.

Cadmium sulfate kills the rainbow trout eggs at 5 ppm and does not appear as toxic as copper sulfate. Our previous tests, however, indicate that trout fry are more susceptible to cadmium sulfate than to copper sulfate. Fry died upon exposure to 5 ppb of cadmium sulfate but approximately 0.1 ppm of copper sulfate was required to kill them under similar conditions.

Leif L. Marking

SECTION OF EFFICACY -- LABORATORY

Intensive screening of fish control agents

Anesthetics

Salmon and trout. The anesthetic combination of MS-222 and quinaldine sulfate was tested extensively during the year on 5 salmonids (Figure 8). MS-222 by itself anesthetized fish rapidly but exposure times must be brief to avoid mortalities. Quinaldine sulfate anesthetizes fish slowly, but affords longer holding times with safety. The mixture then does provide the advantages of quick anesthesia and long holding time.

Table 6.--The toxicity of chemicals to recently spawned eggs from rainbow trout

Chemical	Lethal concentrations (ppm) in exposures of				
	5 days	10 days	15 days	20 days	25 days
Juglone	0.10	0.10	0.07	0.07	0.05
Squaxin	>1.0	>1.0	>1.0	>1.0	0.4
Methyl testosterone	>20	>20	>20	>20	6
Copper sulfate	>10	1.0	1.0	0.4	0.4
Cadmium sulfate	>30	30	30	5	5
Zinc sulfate	>30	>30	>30	20	10

Figure 8.--Charles Ustby checking the effect of the new anesthetic mixture quinaldine sulfate and MS-222 on rainbow trout. Photo taken by Bernard L. Berger.

Mixtures of 20 to 60 ppm of MS-222 with 5 to 20 ppm of quinaldine sulfate demonstrated synergism by being 3- to 5-fold more effective on coho salmon, rainbow, brown, brook and lake trout than the individual components. Fish in concentrations causing complete loss of equilibrium within 3 minutes were exposed for periods up to 60 minutes in waters of 7 to 17 C. with good recovery. Although less chemical is required to anesthetize salmonids at increased water temperatures, total safe exposure time is reduced. Rapid anesthesia, or loss of reflex within 2 minutes in rainbow trout requires a 50:20 ppm of MS-222:$QdSO_4$ mixture.

Water hardnesses from 12 to 350 ppm of calcium carbonate had little influence on the anesthetic properties of MS-222:$QdSO_4$ to rainbow trout. However, in very soft waters the pH is lowered markedly by the addition of efficacious concentrations of the mixture which in turn render the solution less effective on fish. Buffering the solutions back to pH 7.0-7.4 with sodium bicarbonate produces a normal response.

In late fall, MS-222:$QdSO_4$ was employed in practical applications at 5 State and National fish hatcheries. The anesthetic combination was incorporated into the spawning operations on coho salmon, rainbow, brown, brook and lake trout. A desired effect was moderate to rapid anesthesia within 3 minutes. Efficacious concentrations ranged from 20 to 40 ppm of MS-222 in combination with 5 to 10 ppm of quinaldine sulfate to render the spawners manageable (Table 7). Adult lake and brook trout required the highest concentration of MS-222:$QdSO_4$ (40:10 ppm) for adequate knockdown while coho salmon succumbed at 20:10 ppm. Recovery of all salmonids occurred within 2.5 to 8.0 minutes in fresh water and the majority of the fish were swimming upright within 4 minutes.

A 20:10 ppm mixture of MS-222:$QdSO_4$ was tested on 4-inch lake trout at the Jordan River National Fish Hatchery in Michigan. The objective was to determine the value of the anesthetic mixture in large-scale, fin-clipping operations.

Table 7.--Concentrations of MS-222 and quinaldine sulfate, that singly or in combination, anesthetize spawning salmonids to loss of reflex within 3 minutes.

Species and location	Temperature (°F.)	Number of fish	Average weight (lbs.)	MS-222 (ppm)	$QdSO_4$ (ppm)	MS-222: $QdSO_4$ (ppm)
Coho salmon DNR, Platte River, Michigan	42	46	8.0	---	20	20:10
Rainbow trout NFH, Manchester, Iowa	48	74	7.0	80-100	25	30:10
Brown Trout NFH, Manchester, Iowa	47	62	5.7	80-100	25	30:5
Brook trout DNR, Osceola, Wisconsin	48	57	2.1	100-100	25	40:10
Lake trout DNR, Altura, Minnesota	47	55	5.5	---	25	40:10

The fish reached loss of equilibrium within 30 seconds, a desirable characteristic in fin clipping, and fish exposed to the solution for 30 minutes suffered no mortalities.

Samples of muscle tissue were collected from fish exposed to the anesthetics in the laboratory and in the field. In most cases, the fish were exposed for 5.5 and 15 minutes to efficacious concentrations of quinaldine and the mixture of MS-222 and quinaldine, and the tissues were collected after 0, 1, 2, 4, 8, and 24 hours of withdrawal. On occasion, a 48-hour withdrawal sample was taken. The tissues were analyzed for anesthetic residues at Warm Springs.

Warmwater fish. In preliminary testing of the anesthetics on warmwater species including northern pike, carp, black bullhead, channel catfish, bluegill, largemouth bass and walleye, species require 50:10 to 75:20 ratios of MS-222: $QdSO_4$. This compares to a concentration of 150 to 200 ppm of MS-222 when used by itself. Again, the duration of permissible exposure is increased when using the mixture.

Toxicants

Juglone was advanced in the research program because of its potential in eradicating black bullheads. We tested it in outdoor pools on 11 fish species. A concentration of 0.4 ppm was the minimum amount of juglone necessary to eradicate all target species including the most resistant 12-inch black bullheads. Inactivation of the toxicant was apparent after 3 days because fingerling rainbow trout introduced at that time survived a minimum of 96 hours.

A candidate salicylanilide was evaluated for its potential as a toxicant in high pH waters under outdoor conditions. The chemical was applied to 11 fish species at concentrations up to 60 ppb at pH 8.5. It worked well at water temperatures as low as 40° F. Black bullheads along with all other fish were killed in 48 hours by 40 ppb. No mortality occurred among fingerling rainbow trout that were introduced into the pools three weeks after the start of the experiments.

The potentiation of antimycin with naled was investigated further. In outdoor trials, 2 to 3 ppm of naled plus 10 ppm of antimycin were required to eradicate resistant fish in warm, hard and high pH waters. The high concentrations presented solubility problems, and lack of analytical techniques makes further testing of the combination doubtful.

Collecting aid

Propoxate was tested in outdoor, vinyl pools to determine its potential as a fish collecting aid. Stupefaction, slow recovery, inverted position, and settling to the bottom were typical responses of the fish. No repellency was observed. A concentration of 2.5 ppm was efficacious on salmonids, minnows, catfishes and centrarchids and compared well with laboratory findings. A 5-minute exposure rendered most fish immobile; however, goldfish and bluegill required 20 minutes. Fish exposed for 5 minutes recovered in 30-45 minutes when placed in fresh water.

Propoxate is affected little by high pH, e.g., 2.5 ppm in pH 8.5 well water remained toxic to rainbow trout fingerlings for 3 weeks.

Bernard L. Berger

Lethal doses of antimycin

The oral and injected lethal doses (LD50) of antimycin to rainbow trout were determined. Two groups of adult rainbows ranging in weight from 400 to 600 grams and 13.1 to 13.9 inches in length were used as test animals. One group of fish received selected oral doses of antimycin in gelatin capsules, and the second group received intraperitoneal (ip) injections of a 1 ml. ethanol-water solution containing selected doses of antimycin. After dosing, the fish were placed in flowing well water for observation. Results were as follows:

Oral toxicity. The oral, 48-hour LD50 of antimycin to adult rainbow trout was 2.50 mg/kg with a 95-percent confidence interval of 0.76-8.25 mg/kg.

Injected toxicity. The ip 48-hour LD50 of antimycin to adult rainbow trout was 0.105 mg/kg with a 95-percent confidence interval of 0.078-0.140 mg/kg.

Judging from the bioassay immersion toxicity data accumulated at this laboratory, the actual dose of antimycin required to kill rainbow trout is in terms of µg/kg. The differences between immersion, oral and ip toxicity further illustrate the efficiency and importance of the gill and the role it plays in the entry of chemicals.

Wayne A. Willford

SECTION OF EFFICACY -- FIELD

Evaluation of fish control agents in the field

Minimum contact time

Control of fish populations with toxicants requires that the target species be in contact with the chemical for a specific length of time. Concentration and contact time are equally important. In treating a stream, the bolt of toxicant moves downstream and the fish are exposed for the length of time it takes the bolt to pass any particular point in the stream. Thus, we must know how long an exposure to a given concentration is needed to eliminate the target species with a particular toxicant.

We conducted tests in flowing water in a stainless steel trough to determine the minimum contact time to eliminate 100 percent of selected species of fish with antimycin and rotenone. The water had a total hardness of 220 to 250 ppm and pH of 7.7 to 7.9. The temperature was held constant at either 12 or 17 C., ± 1 C. We applied the chemical to the entering flow of 29 liters of water per minute. Groups of fish were moved to another trough with flowing, fresh water after selected periods of exposure in the treated trough, and observed for approximately 96 hours.

At 12 C., the minimum contact time for 100 percent mortality was much less with antimycin than with rotenone (Table 8). This was especially true for carp and white suckers which are often target species. The concentrations of rotenone recommended by the manufacturer required up to 24 hours of exposure to kill carp whereas recommended rates of antimycin took only 6 hours. The exposure required for rotenone in water at 17 C. was 50 to 67 percent less than that needed at 12 C.

Rotenone is generally considered a fast-acting toxicant. This is true if only initial response is the criterion because fish do exhibit distress and lose equilibrium rapidly. However, fish exposed to rotenone in these tests laid on their sides up to 12 hours or more but recovered when placed in fresh water. Conversely, many fish exposed to antimycin showed no distress when placed in fresh water, but died within 24 hours. In no case did a fish recover after exhibiting distress for antimycin.

Solid formulation of antimycin

Major problems in treatment of streams with toxicants are the application of constant concentrations and the manpower needed to maintain the application apparatus.

Ayerst Laboratories has produced experimental, solid-block formulations of antimycin designed to dissolve at a constant rate and eliminate the need for constant observation of application equipment. The blocks, which look like large chocolate bars, are composed of antimycin, sodium fluorescein, and a surface active agent. The sodium fluorescein inhibits the formation of antimycin crystals as the toxicant dissolves from the bar.

The formulations were tested in a river in central Wisconsin. Some bars dissolved at a constant rate and effectively eliminated caged carp in the treatment area. In blocks that did not contain enough sodium fluorescein, the antimycin formed a thick lattice of crystals around the bar, keeping it from further dissolution after a few hours. Improvements are being made in the formulations based on the results of the field trial. We think this innovation will considerably reduce the cost of manpower and equipment needed for stream treatments.

Philip A. Gilderhus

Table 8.--Number of hours of exposure to antimycin and rotenone required for total mortality of selected species of fish

Species	Temp. (C.)	Hours in antimycin at 10 ppb[1/]	Hours in rotenone at 50 ppb	100 ppb	250[2/] ppb
Rainbow trout	12	1	2	-	-
Carp (small)	12	6	-	-	-
Carp (large)	12	6	24	18	15
	17	-	10	9	5
White sucker	12	4	18	15-17	9
	17	-	9	5	3
Black bullhead	12	-	-	21	15
	17	-	9	-	3
Green sunfish	12	8	8	-	-
Largemouth bass	12	6	8	-	-
Yellow perch	12	2	-	-	-

1/ Fintrol-Concentrate (10 percent antimycin) expressed as active ingredient.

2/ Nox-Fish (5 percent rotenone) expressed as active ingredient.

Toxicants against Tilapia and Clarias

On-site bioassays of antimycin and rotenone were made in Florida against Tilapia aurea and T. Mossambica of 2 to 5 inches long. The species are more resistant to antimycin than largemouth bass and bluegills.

Attempts to bioassay antimycin against the walking catfish (Clarias batrachus) in Florida were thwarted by insufficient numbers of fish. Instead, two small ponds were treated with 50 and 150 ppb of antimycin to determine if the walking catfish would attempt to escape as they reportedly do from rotenone applications. The species is as resistant to antimycin as bullheads, and only a few 3- to 10-inch specimens were killed by 150 ppb. None of the dying fish, however, tried to leave the pond.

Ralph M. Burress

SECTION OF FISH PHYSIOLOGY

Effect of fish control agents on blood chemistry and hematopoietic tissue of fish

Antimycin is known to exert its biochemical effects at the subcellular level by blocking the electron transport chain. In these studies (Table 9), fish exposed to lethal concentrations of antimycin die in an acidotic state. The blocking of oxidative phosphorlyation causes a buildup of lactic acid and glucose in the plasma, reduces buffering capacity of the blood (as measured by the total plasma CO_2), and drops the pH of the blood to lethal levels (Figure 9).

Richard A. Schoettger

Table 9.--Effects of antimycin poisoning on blood pH and plasma total CO_2, glucose and lactic acid concentrations in rainbow trout, channel catfish and black bullhead.

Species	Conc. of antimycin (ppb)	Exposure time (hrs)	No. of fish	pH mean	pH ± S.D.	CO_2 (vol. %) mean	CO_2 ± S.D.	glucose (mg %) mean	glucose ± S.D.	lactic acid (mg %) mean	lactic acid ± S.D.
Rainbow trout	control	-	11	7.42	0.12	26.3	3.7	105	34	43	39
	2	1	5	7.25	0.10	19.4	2.2	122	14	118	53
	2	2	5	7.36	0.84	23.2	1.9	121	32	59	24
	2	3	5	7.40	0.84	18.5	4.8	129	55	103	20
	2	4	5	7.45	0.09	18.6	3.5	125	32	97	21
	2	5	5	7.44	0.55	13.9	1.9	71	38	132	14
	2	6	5	7.40	0.10	14.2	4.0	120	34	103	27
	2	7	3	6.88	0.13	8.9	1.3	184	27	164	19
Channel catfish	control	-	9	7.54	0.02	22.5	3.8	65	24	14	3
	2	1	2	7.67	0.23	24.4	2.1	179	32	-1/	-
	2	3	2	7.51	0.15	18.5	2.7	86	21	-1/	-
	2	5	2	7.74	0.52	19.4	3.1	89	27	-1/	-
	2	7	2	7.62	0.47	20.0	1.9	80	23	-1/	-
	80	1	5	7.45	0.10	13.2	1.7	96	35	39	5
	80	2	5	7.16	0.45	7.1	1.1	170	64	93	7
	80	2½	2	7.05	0.32	8.5	1.4	174	41	92	3
	80	3	5	7.04	0.55	6.9	1.7	200	33	98	4
Black bullhead	control	-	5	7.69	0.13	19.8	2.1	45	23	56	43
	200	1	5	7.38	0.24	14.8	4.4	91	42	54	40
	200	2	5	7.12	0.13	9.9	4.9	59	30	63	52
	200	3	5	7.23	0.23	8.5	3.6	106	68	59	30
	200	4	5	7.27	0.28	10.5	6.1	145	64	72	52

1/ Samples contaminated during analysis.

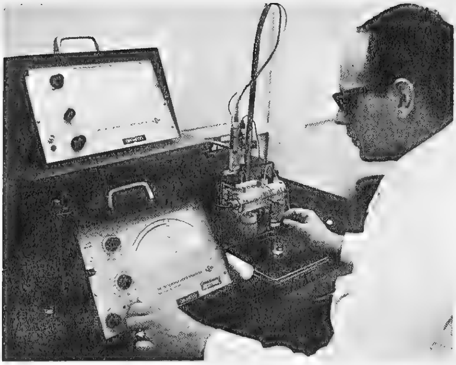


Figure 9.--Chemist Wayne A. Willford utilizing blood gas analyzer for measurement of blood pH and dissolved gases. Photo by Bernard L. Berger.

Effect of fish control agents on the central nervous system

From previous studies we have shown that MS-222 anesthesia disrupts, either directly or indirectly, the in vivo cationic equilibria in the brain of rainbow trout. In an attempt to determine if these electrolyte changes are peculiar to MS-222, we tested two additional anesthetics, quinaldine and methylpentynol, using the same experimental design.

Similar changes in brain electrolytes occur with all three anesthetics. The most significant of these changes are a progressive loss of K^+ accompanied by a progressive increase of Fe^{3+} in the brain during the initial 2 minutes of exposure to the anesthetics. Longer exposures result in a return of brain K^+ and Fe^{3+} towards control values. Concentrations of anesthetics used in this study also produced the greatest behavioral changes within the initial 2 minutes and little change occurring after this period. Thus, the pattern of change in electrolyte content may be associated with the knockdown time of the particular anesthetic.

The degree of electrolyte change also appears to be associated with the anesthetic state of the fish. MS-222, which produces the deepest anesthetic condition in fish, also produces the largest change in electrolyte content of the brain. The changes which resulted from exposure to quinaldine ranked second in intensity, and methylpentynol the smallest change in brain electrolytes.

Wayne A. Willford

Effects of fish control agents on behavior of fish

Cooperative studies with the Psychology Department, Wisconsin State University-La Crosse, on the influence of quinaldine on the rate of conditioning in bluegills have shown no differences between control and quinaldine-exposed fish. One consistent observation was the wide variation in individual learning ability in both quinaldine-treated and control groups.

Richard A. Schoettger

Effects of fish control on the reproductive system of fish

An attempt was made to sterilize bluegills by exposing them to diethylstilbestrol. We used younger fish and a more water soluble form of the chemical than in our previous tests. The 6-month-old bluegills averaged 1.7 inches in length and 1.3 grams in weight. They were exposed for 10 days to a solution of the sodium salt of diethylstilbestrol diphosphate containing 0.3 ppm of diethylstilbestrol. The fish were held in a raceway from January to May and then in outdoor, 0.01-acre pools from June to November. Three pools contained treated fish, and three with untreated fish served as controls.

There was a considerable amount of natural reproduction in two of the three pools containing treated fish. Reproductive success appears to have been related to the ecology of the individual pools rather than the treatment of the fish before they were placed in the pools. Any further experiments with diethylstilbestrol will be done with fish in their first 60 days of life, i.e., at an earlier stage of gonadal development.

Philip A. Gilderhus

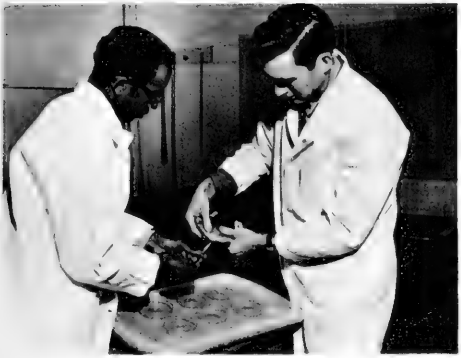


Figure 10.--James Hixson and Les Chew collecting sperm for fertilization of eggs used in the bluegill sex reversal study. Photo by photographer, Warm Springs, Georgia Foundation.

Attempts at sex reversal in bluegills by feeding methyl testosterone were continued at Warm Springs (Figure 10). All fish involved in last year's tests were sexed. The lot which received medicated feed for the first 60, post-hatch days at the rates of 10, 30, and 50 micrograms per gram of feed was the only lot that may contain sex-reversed males. Six males from this lot spawned in plastic pools, and progeny grew well. Next spring, when the fingerlings have reached a desirable size, they will be sexed and the results of this phase of the experiment evaluated.

There were few survivors in some of last year's tests, and one lot of bluegills was given feed containing 0, 25, and 50 micrograms of methyl testosterone per gram of feed for the first 30, post-hatch days. These rates of medication were about equal to those used last year, and the period of treatment spanned the life stage at which survival had been lowest. Survival was good this year, and there was good growth before cold weather.

No attempts had been made to treat fry before they were old enough to feed, and this could be the time sex direction occurs, so 4 lots of eggs and the resultant fry were treated with 1, 2.5, and 5 ppm of methyl testosterone in a water bath for 7 days after the eggs were fertilized. Some fry survived both the 1 ppm and 2.5 ppm treatments. They were placed in plastic pools where good growth was attained.

Fry from another lot were divided into three tanks, and received medicated feed for the first 30 days at the increased rates of 100, 250, and 500 micrograms of methyl testosterone per gram of feed. Still another lot was fed at identical rates from the thirtieth to the sixtieth days of life. Survival was good in all lots that received medicated feed this year, and good growth was attained by late fall.

Leslie E. Chew

The fate of control agents in fish

We previously determined (1968 Annual Report) that MS-222 concentrated in the brains of rainbow trout exposed to a 100 ppm solution at 12 C. In recent studies on rainbow trout anesthetized in a solution containing a mixture of MS-222-quinaldine sulfate (30:5 ppm) for 15 minutes at 7, 12, and 17 C. MS-222 indeed concentrated in the brain (brain to blood ratios of 2.1, 2.1, and 2.6, respectively) while the blood concentration was that of the anesthetizing solution. MS-222 residues were rapidly cleared from the brain during the first hour of recovery.

Some 320 quinaldine residue analyses have been performed on five salmonids anesthetized with quinaldine sulfate, or a mixture of MS-222 and quinaldine sulfate. At efficacious concentrations, the quinaldine residue level in all species is around 0.01 ppm at the end of 8 hours withdrawal and 0 after 24 hours (Figure 11).

Some 220 MS-222 residue analyses have been performed on five salmonids anesthetized with MS-222 or the combination anesthetic by colorimetric analysis. Confirmatory analysis by thin layer chromatography is in progress. At efficacious concentrations, the MS-222 residue level in all species after 24 hours withdrawal is within the background reading of the control fish (Figure 12).

John L. Allen

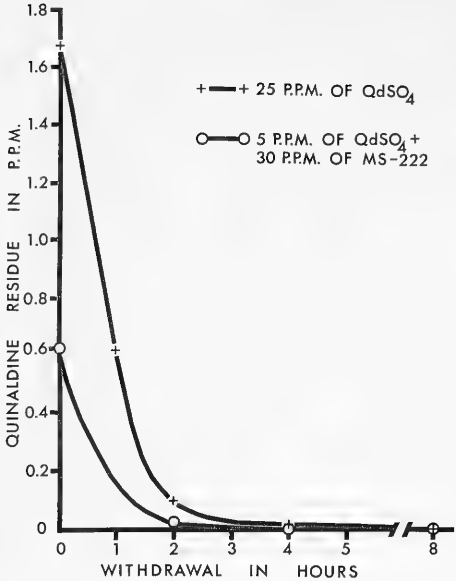


Figure 11.--Quinaldine residue in the muscle of brown trout at various withdrawals after anesthetization in 25 ppm of quinaldine sulfate or a mixture of 5 ppm of quinaldine sulfate with 30 ppm of MS-222.

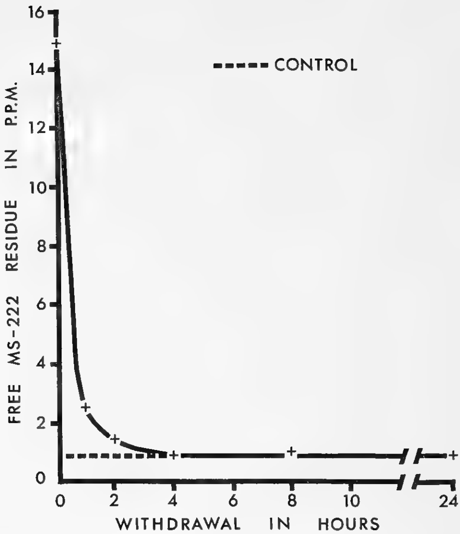


Figure 12.--Free MS-222 residue in brown trout muscle at various withdrawals after anesthetization in a mixture of 5 ppm of quinaldine sulfate and 30 ppm of MS-222.

Effects of fish control agents on the renal system of fish

Anesthetics are often used in handling experimental animals. MS-222 is reported to affect Na and H_2O balance in Bufo marinus following anesthesia. We have detected similar effects in rainbow trout by urinalysis following MS-222 anesthesia. Nine trout were anesthetized in a 100-mg/l solution, catheterized and placed into a urine collecting apparatus. These fish were allowed to recover for 18 to 20 hours before collection of a 3-hour urine sample which served as a control. Then, the trout were re-anesthetized in the collecting apparatus with a 100-mg/l solution for 5 minutes. Urinanalysis was made on samples of accumulated urine collected 2, 4, 6, 8, and 12 hours post-anesthesia (Table 10). Urine output (ml/kg/day) is increased following MS-222 anesthesia. Loss of inorganic ions also increases and the Na concentration in the urine parallels the urine flow pattern. Most ion concentrations return to control levels within 12 to 24 hours of recovery.

Joseph B. Hunn

Development of methods related to fish controls

Control of pH

Because water quality and pH in particular contribute to the inactivation of chemicals and because many natural waters are alkaline, we have developed chemical buffering formulations to simulate problem water (Table 11). Most chemical buffers are toxic to fish at high concentrations; the amounts suggested in the literature were modified to accommodate the fish. The buffering materials and quantities are specifically for our standard reconstituted water of about 44 ppm in total hardness, and they may require alterations in other waters. These buffers maintain the pH within 0.2 of a unit with minor

Table 10.--Characteristics of urine from rainbow trout following re-anesthetization in a 100-mg/1 solution of MS-222 for 5 minutes at 12 C.

Urinary parameter	Units	Control	Time in hrs. post-anesthesia				
		3	2	4	6	8	12
Flow	ml/kg/day	43.8(6)1/ 16.1-92.2 2/	139.0(9) 29.0-234.8	114.9(8) 58.1-200.0	73.3(8) 17.9-120.0	92.6(8) 29.0-163.3	87.1(6) 44.8-126.7
Na^+	mequiv./1	4.3(7) 2.2-8.7	8.4(9) 3.5-15.7	5.4(8) 3.0-9.9	5.1(8) 2.2-9.9	4.1(7) 2.0-7.0	3.6(6) 1.0-6.4
K^+	mequiv./1	1.4(7) 1.3-1.5	1.5(9) 1.1-1.9	1.5(8) 1.2-1.8	1.3(8) 1.2-1.7	1.2(7) 1.1-1.3	1.2(6) 0.8-1.4
Ca^{2+}	mequiv./1	2.9(7) 1.4-3.9	3.2(9) 1.2-4.6	3.2(8) 2.3-4.2	3.0(8) 2.0-4.7	3.1(7) 2.2-5.3	3.6(6) 1.6-7.9
Mg^{2+}	mequiv./1	3.0(7) 1.3-5.8	2.7(9) 1.5-6.2	2.9(8) 0.8-7.9	4.1(8) 1.5-8.9	3.9(7) 1.4-13.3	2.6(6) 0.8-6.7
Zn^{2+}	mequiv./1	0.2(4)3/ 0.1-0.4	0.4(7)3/ 0.1-1.2	1.2(6)3/ 0.2-2.8	1.3(4)3/ 0.5-3.1	0.5(5)3/ 0.1-1.0	1.4(4)3/ 0.8-2.4
Cl^-	mequiv./1	5.1(7) 2.0-11.7	6.0(9) 0.7-16.9	3.8(8) 0.9-9.1	6.1(8) 0.9-12.8	5.7(8) 0.9-18.4	3.0(6) 0.9-9.7
PO_4 as P	mM/1	2.5(7) 0.3-5.2	4.8(9) 1.0-13.1	3.7(8) 0.0-9.6	3.5(8) 0.3-9.7	2.8(8) 0.3-8.9	3.7(6) 0.5-7.4

1/ Mean value with number of samples in parentheses.

2/ Range.

3/ Concentration x 10^{-2}.

Table 11.--Buffer chemicals for maintaining pH in bioassays

pH	Ml. of solutions for 15 l. of water		
	1N NaOH	1M KH_2PO_4	0.5M H_3BO_3
6.0	1.3	80.0	----
6.5	10.0	30.0	----
7.0	19.0	30.0	----
7.5	----	----	----
8.0	19.0	20.0	----
8.5	12.0	11.5	----
9.0	8.8	----	30.0
9.5	11.0	----	20.0
10.0	16.0	----	18.0

daily adjustments, and we determined they are not toxic to fish. Bluegills survive well in buffered water from pH 6 to 10, whereas rainbow trout require special acclimation prior to being placed in high or low pH water.

Leif L. Marking

The addition of MS-222 to soft water depresses the pH of the anesthetic solution, and fish placed in it show signs of irritation. Stress and irritation can be alleviated by adding a buffer to raise the pH.

The efficacy of quinaldine as an anesthetic is greatly influenced by pH. The efficacy appears to be directly related to the concentration of the lipid-soluble free base of quinaldine. The quinaldine ion and quinaldine free base concentrations at a given pH can be calculated from the published pKa value of quinaldine (5.42, 5.8).

John L. Allen

DO in vinyl pools

We have observed sharp losses of dissolved oxygen, from 7.5 ppm to >1.0 ppm, in new 1,000-gallon polyvinyl pools immediately after set-up. The loss may be caused by plasticizers in the vinyl liner. To overcome the problem, we made polyethylene liners and compared them with polyvinyl liners by filling both with well water and making daily O_2 measurements. After 9 days, water in the polyethylene pools had 7 or more ppm of dissolved oxygen in contrast with 3.1 ppm of DO in the polyvinyl pools. Black polyethylene liners demonstrated an ability to hold cooler temperatures in the summer.

Bernard L. Berger

Automatic data processing

We have cooperated with the Division of Pesticides Registration to develop a storage and retrieval system for toxicity data. Our data, principally LC_{50}'s, are summarized on abstractor cards identifying chemical structure, test organism, test system, and references according to codes previously designed. The cards are submitted for automatic data processing (ADP). The data generated by cooperating agencies are useful to us, to other researchers, and to chemical registration.

Leif L. Marking

Dispersion of chemicals in streams

The most convenient way to predict the behavior of a bolt of chemical in a stream is to apply a tracer material such as salt or fluorescent dye and measure the buildup and decline of concentrations at different points on the stream. Whereas the tracer dyes are readily available, there is no accurate method for conducting the dye study, interpreting the results, or applying the results to compensate for losses of concentration and time.

Studies have now been conducted in two streams to determine how long a tracer dye must be applied to give a true picture of the dispersion of an extended application of toxicant.

Several factors have become evident in our studies. At any point on a stream, the concentration of dye builds from zero to a peak somewhat gradually. The time required for the buildup is governed by the amount of stored water in the stream between application point and sampling point. The more stored water there is in pools, the longer the buildup time. In turn, the longer the buildup time, the shorter the time that the peak concentration is maintained. At any given concentration, the chemical must be applied for long enough to saturate the stored water before a true peak can be reached.

In each stream, a brief application of dye (rhodamine-B) gave a much lower peak than a longer application in the same stretch of stream. For example, in one stream, a 15-minute application of dye at 15 ppb gave an instantaneous peak of 2.6 ppb at the sampling point. A 4-hour application of 15 ppb gave a peak of 9.5 ppb which lasted 2 hours. If the short bolt of dye had been used in preparation for a reclamation of the stream, it would have indicated a much greater loss of both concentration and time than actually the case. Thus, the amount of toxicant then applied to compensate for the losses would be several times the amount needed.

To obtain a true estimate of the peak concentration, a tracer must be applied long enough to give a flat peak of at least 30 minutes duration at the sampling point.

Dispersion of chemicals in lakes

The surface waters of lakes, down to depths of about 20 feet, are comparatively easy to reclaim, especially with the newer granular formulations which release the toxicant evenly as they sink. Several States, however, need to treat lakes to depths of 100 feet or more. The only method available at present is to pump a liquid toxicant down through a weighted hose, but the adequacy of the method has not been thoroughly assessed. We therefore cooperated with the State of Minnesota to measure dispersion of a liquid toxicant in deep waters of a small lake.

Taylor Lake has an area of 54.8 acres and a maximum depth of 84 feet. The upper 15 feet of water were treated with sand formulation antimycin. The water between 15 and 35 feet of depth was treated with liquid formulation antimycin to which rhodamine-B dye had been added. The amount applied was calculated to give 1 ppb of antimycin and 10 ppb of the dye in the 15 to 35 foot stratum. The liquid was pumped through a single hose at various depths in that stratum as the boat traversed a grid pattern. Water samples were taken at selected depths in the stratum, and the concentration of dye was analyzed with a fluorometer.

Only 30 percent of the samples taken 24 and 48 hours after treatment contained detectable amounts of dye. Thus, 70 percent of the water in the stratum contained inadequate concentrations of toxicant. Some samples contained over 60 ppb of dye--this indicated the formulation had not moved much from the paths traveled by the hose.

Seven days after application, the deep water was sampled again. All of the samples between 15 and 40 feet contained detectable amounts of dye, but 33 percent contained less than 5 ppb of dye or 0.5 ppb of antimycin.

There is, therefore, some question whether a toxicant will remain biologically active until it becomes completely circulated or dispersed in deep water. In this lake, however, all of the target fish were killed including large white suckers and perch--they probably were in the warm, top 15 feet of water. There is a real need for better methods of treating deep water.

Philip A. Gilderhus

Water analysis

Cooperative studies with Hatchery Biologists at Genoa NFH to evaluate methods of water analysis resulted in both stations converting their methods of analysis for phosphates. Some minor alterations were also instituted in the analysis for ammonia.

Wayne A. Willford

Methods in field bioassays

Development of methods for field bioassays are essential to well executed chemical treatment of lakes and streams. According to laboratory tests of different kinds and sizes of containers made of 6 different materials, glass jars, plastic bags, and plastic waste cans are best for bioassays. Of them, a 75-gallon bag made of clear, 3-mil polyethylene was chosen for further testing as a field bioassay container on the basis of utility, economy, and availability (Figure 13).

Initial tests of the bags in the field were devoted largely to comparisons of water quality in ponds and bags. The bags appear to be entirely adequate for bioassay purposes, and several methods for filling, suspending, and protecting them were developed.

Three ponds were treated with antimycin on the basis of bioassay results. Subsequent evaluation of the kills afforded a good basis for determining concentrations required for fish eradication. Additional tests will have to be made under a variety of conditions to further develop the method and to find suitable techniques for choosing concentrations required for selective control of fish populations.

Ralph M. Burress

Analytical methods for residues of fish control agents

A thin layer chromatographic method of identification of quinaldine may be applicable to residues in fish. A positive test for quinaldine in extracts containing 0.1 ppm of quinaldine was indicated by gas chromatography in preliminary investigations. The method consists of spotting the extracts on an alumina TLC plate, and developing the plate with 1 percent ethyl acetate in iso-octane. The plates are examined under long wave ultraviolet light after spraying with concentrated sulfuric acid and heating. The use of filters transmitting light from 415-490 mμ decreases the background fluorescence in fish extracts.

John L. Allen

RESEARCH FISH

We received many requests from outside agencies for test fish during 1969. Among them were the University of Minnesota, the Hormel Institute at Austin, Minn., Winona State College, the Hydrobiology Station at Winona, Minn., Hatchery Biologists at Genoa NFH, and the chemistry and biology departments at Wisconsin State University-La Crosse.

Our main sources of test fish for La Crosse were the National fish hatcheries, supplemented with valuable contributions from the fishery departments of Wisconsin, Iowa and Minnesota. We received late spring and early fall rainbow trout eggs from Troutlodge Springs. We had good cooperation with all agencies.

Figure 13.--Biologist Ralph Burress and Biological Aid Jerry Moncrief conducting an on-site bioassay using anchored 70-gal. plastic bags that are surrounded by a seine enclosure. Photo by photographer, Warm Springs Georgia Foundation.

Bioassay-size fish included bowfin, coho salmon (both Pacific and Lake Michigan strains), rainbow, brown, book, and lake trout, goldfish carp, fathead minnow, golden shiner, white sucker, channel catfish, black bullhead, madtom, central mudminnow, largemouth bass, green sunfish, bluegill, yellow perch and walleye. Eggs from coho salmon, rainbow trout, lake trout, and white sucker were used. We spawned some rainbow trout to furnish green eggs for the testing programs. More than usual numbers of large and medium sizes of rainbow, brown, brook, and lake trout were used in the anesthetic testing program. We also acquired other large fish, including shortnose gar, northern pike, and buffalo.

Commercial pellets, Oregon moist pellets, ground beef liver, and frozen brine shrimp are used as feed. We raise live daphnia for feeding small fish the year around and maintain the culture during winter in the large public aquarium tanks. Small minnows from bait dealers furnish live forage for large northern pike, bass, bluegills and others.

External parasites are the most troublesome disease problems. Anchor worms (Lernaea) have been almost eliminated from 1969 shipments of carp and goldfish. Most other external parasites can be held in check, if not eliminated, with formalin treatments. Ichthyophthirius continues to be a major source of trouble. Warm water (85-95° F.), fast flowing shallow water, and frequent formalin flushes, used separately or in combination, depending on the species infested or the amount of infestation, have all been used to eradicate ich. In general, prevention is better than cure.

An interesting development was the sudden appearance of furunculosis in green sunfish and mudminnow which had arrived and been held here in a healthy condition for some time. Contagion was traced to brown and rainbow trout which appeared to be healthy but were carriers. Bacterial gill disease is the most often encountered trouble in small trout. Rust precipitate in the water, crowding, or holding the feed level down to prevent fast growth can all bring on an outbreak of B. G. D. If caught in time, Roccal treatments and a few days of liver feeding will usually control the disease. Internal parasites and columnaris in black bullheads, fungus infections on carp and goldfish, and an internal bacterial disease in white suckers were also encountered. Several lots of different species were discarded when we judged they were not worth treating.

Everett W. Whealdon

The Marion and Warm Springs National Fish Hatcheries were most cooperative and supplied most of the fish that we used at Warm Springs this year. One lot of rainbow trout was acquired from the Cooperative Fishery Unit at Auburn, Ala.

Largemouth bass, bluegills, and channel catfish were the most used species of fish, and some were on hand the entire year. Other species of fish used included rainbow trout, goldfish, golden shiners, black bullhead, brown bullhead, striped bass, warmouth, green sunfish, and redear sunfish.

Prophylactic treatments of salt, acriflavin, or formalin were administered to all fish shortly after arrival and occasionally throughout the year to prevent outbreaks of disease and parasites. One lot of channel catfish infested with Ichthyophthirius was cleaned up by a daily flush treatment of formalin (1:4000) for 4 weeks. Mortality from columnaris was arrested in one lot of bluegills and one lot of black bullheads by a 72-hour treatment with 15 ppm of Terramycin.

Commercial pellets were fed to most of our fish three times a week. This was supplemented occasionally with fish or liver. The small bluegills involved in the sex reversal experiments received some daphnia. Large bluegills in this experiment were fed some red worms and grass shrimp with the dry trout pellets.

Leslie E. Chew

LIBRARY SERVICES

Bibliographic services were provided on the following topics: fish carcinomas, attractants, repellents, catfish farming, pollution control, pesticides, farm ponds, rotenone, and antimycin. A bibliography on formalin as a fishery tool is nearing completion. Special reports on the serial holdings and the publications of the Laboratory were compiled and distributed. A Library Service Report was submitted to the USDI Library. Our mailing list for publications was validated and expanded to include 3,500 individuals and organizations. Seventy-five books and nearly 700 reprints were acquired and catalogued for the research staff.

Rosalie A. Schnick

FISH HUSBANDRY RESEARCH--TOMORROW OR TODAY?

Remember the graffiti adorning the fences and public monuments a few years ago--the one that said, "Due to lack of interest, tomorrow has been cancelled"? Well, don't apply this to fish husbandry research, because we're moving ahead into the future. Rather, unfold the banner that says, "Because of overwhelming demand, and the success of our program, tomorrow will be held today."

In the following pages are chronicled the things we have been doing in fish husbandry research as "today's" things. The topics look familiar to us; indeed, many of them are enduring investigations which are just now bearing fruit as we make long strides down the research roads to disease, culture, and nutrition knowledge.

Superimposed upon these pursuits of today are some we didn't expect until tomorrow. We have not been able to take the new efforts in stride without some interruption of ongoing research, but have managed to make a mix so the new and the old could both go forward.

One of tomorrow's quests is the catfish-pesticide inquiry in the Southeast. In 1969 a new syndrome began to plague the catfish. Neither the fish farmer nor the fish farmer's friend (our scientists from the Warmwater Fish Cultural Laboratories) knew this catfish condition that had suddenly surfaced. Our people from Stuttgart and Marion, after much study, hypothesized that pesticides, in combination with cold weather, caused the syndrome. A larger program in 1970 will be undertaken to learn more about the new disease.

Another of tomorrow's problems we faced was our deep involvement in educational and extension exercises. True, we have always distributed reprints and answered questions and looked at sick fish, but who would have conjectured, a few years ago, that the entrances to our laboratories would have lineups like that at the West Gate at Yellowstone, or that one laboratory would respond to more than 4,000 written requests for information? These things have unexpectedly been batted to us, and we have fielded them.

Yes, tomorrow is upon us today! The momentum is increasing, but we are ready, for fish husbandry research has not saved today's tasks for tomorrow.

Oliver B. Cope, Chief
Branch of Fish Husbandry Research

EASTERN FISH DISEASE LABORATORY

Leetown, West Virginia
S. F. Snieszko, Director

HIGHLIGHTS

Mr. Bullock spent nearly three months at the Unilever Marine Laboratories, Aberdeen, Scotland, working on bacterial diseases of cultured marine fishes.

The reliability of methods for detection of furunculosis of trouts by bacteriological and serological examination was tested at three National Fish Hatcheries.

Two chapters for the textbook on Fish Diseases were submitted to the publisher. The chapters are "Bacterial Diseases" and "Identification of Fish Pathogenic Bacteria."

Myxosoma cerebralis, the causative agent of whirling disease, is being maintained in the laboratory for control and eradication research. It has been found to survive -20° C. for two months and in an aquarium for over 2 years.

Dr. Wolf went to the Laboratory of Virology, St. Jude Children's Research Hospital, Memphis, Tenn., to update his competence in virology. In collaboration with Dr. R. W. Darlington, staff electromicrocopist, he found the channel catfish virus to be a member of the Herpes virus group, and sequential changes during in vitro infection have been determined with electron microscopy.

Channel catfish virus growth rates, characterization data, and sequential light microscopy of infected cultured cells have been determined. The data indicate possible control measures for the disease.

Culture dish plaque assay of channel catfish virus has been developed for use in normal atmosphere; this obviates the need for CO_2 incubators and provides a sensitive, economical and accurate virological tool. Early trials have shown that salmonid viruses can be similarly plaqued.

Dr. Tokuo Sano, visiting scientist, isolated IPN virus from glycerol-preserved trout fry sent here from epizootics among Japanese hatcheries.

Caudal fin erosion in Dover sole

While in Scotland, I learned that one of the problems in Dover sole culture is low but persistent mortality from caudal fin erosion. Previously, I had investigated a similar condition in hatchery-reared brook trout; therefore, I undertook a study of the disease in Dover sole. The main questions to be answered were whether one or more species of bacteria are involved and, if so, what treatment might be used. Results of the bacteriological examination of 15 sole with caudal fin erosion were remarkably like those obtained with the brook trout. Several species of aquatic bacteria were isolated from the eroded tail fin but none could be isolated from internal organs. Therefore, control of the erosion might be accomplished by use of external disinfectants. To begin with, tests were conducted to determine the toxicity of three quaternary ammonium compounds for the sole and then to determine the in vitro sensitivity of the isolated bacterial strains to the same compound. Three benzalkonium chlorides were used: Hyamine 3500, Hyamine 1622, and Hyamine 2389. The sole tolerated all compounds at 1 and 2 ppm for an hour, with no residual toxicity noted after 24 hours. The 3 compounds were also tolerated at 3 and 4 ppm for 1 hour but some sole died within 24 hours after treatment in Hyamine 3500. Therefore, the Hyamines appeared to be of possible prophylactic or therapeutic use in controlling tail fin erosion.

G. L. Bullock

Cultural characteristics of myxobacteria pathogenic to fish

Study was continued on myxobacterioses, especially gill disease, and including myxobacteria from Dr. Ian Anderson, Unilever Research Laboratory. Cultures were isolated from gill disease in Atlantic salmon and from a condition termed "eroded mouth" in rainbow trout raised in sea water. Earlier results here indicated that some characteristics of myxobacteria from gill disease were fairly homogeneous. Therefore, we were anxious to compare gill disease cultures from Scotland with our strains. Morphological and physiological characteristics of our old stock cultures were rechecked and characteristics of the new isolates determined.

Myxobacteria from all sources were proteolytic, produced amylase, lysed intact A. liquefaciens cells, and grew from 5°-30° C. They were variable in ability to produce acid from glucose, reduce nitrate, and degrade tyrosine. Also, there was a variety of morphological colony types among cultures from all sources. These observations showed that myxobacteria in gill disease are more diversified than expected. For serological reactions, 9 rabbit antisera were prepared against myxobacteria isolated from gill disease and eroded mouth in Scotland, gill disease in bluegills, and tail rot in our hatchery brook trout.

G. L. Bullock and H. M. Stuckey

Serological tests for diagnosis of bacterial fish diseases

Over the past 8 years we have concentrated on methods for rapid and accurate diagnosis of bacterial fish diseases. We have progressed from morphological and biochemical methods to the more rapid agglutinin and precipitin tests. One of the most rapid serological tools for diagnosing infectious diseases indirectly from infected tissue is the direct or indirect fluorescent antibody technique. This has been used in human and veterinary medicine for some time and recently Klontz used it in studying serotypes of Aeromonas salmonicida and for demonstrating the presence of A. salmonicida in wild populations of fish.

We investigated the indirect fluorescent antibody technique for identification and differentiation of A. salmonicida, A. liquefaciens, and Pseudomonas fluorescens in infected fish tissues. Stock cultures of the three organisms were studied first to detect cross reactions among the three types, especially between strains of A. liquefaciens and A. salmonicida. We hoped the strains of the three bacteria would react with only the antiserum prepared for the particular species, but cross reactions occurred between strains of A. salmonicida and A. liquefaciens. No cross reactions were noted with the P. fluorescens strains and the two aeromonad antisera. Cross reactions were virtually eliminated between the aeromonad species by cross absorbing the two sera.

Survey of trouts at three National Fish Hatcheries for the presence of furunculosis and kidney disease

The proposed classification of the National Fish Hatcheries as to the presence or absence of furunculosis, kidney disease, whirling disease, infectious pancreatic necrosis (IPN), and viral hemorrhagic septicemia (VHS) has raised questions as to the best methods of detecting these diseases, especially in the latent or carrier state. Adequate methods already exist for IPN, but not for the other diseases. Since a survey for these diseases was to be made in the brood stock hatcheries in Region 5, we decided to test different methods of detection of A. salmonicida, the causative agent of furunculosis. We also examined trout for the presence of antibodies against this bacterium.

We chose furunculosis rather than kidney disease because the kidney disease corynebacterium is still very difficult to grow.

We examined trout at Cortland and White Sulphur Springs, which had no known furunculosis, and from Bowden where furunculosis was known to be present. To avoid killing yearling and adult trout, blood was removed aseptically from the hemal canal and cultured in an attempt to isolate A. salmonicida. Tryptic soy agar (TSA) slants were inoculated with 0.5 ml of blood overlaid with 2 ml of nutrient broth containing 0.01 percent heparin. The reason for using a two-phase medium was to increase the chance of isolating very low numbers of bacteria. For comparison, kidney material was also cultured from approximately 20 percent of all yearling and adult trout. We cultured only kidney material from fingerlings because we could not obtain enough blood for culture. A small quantity of blood was obtained from fingerlings. Serum and plasma samples were tested by means of slide agglutination test for the presence of antibodies against A. salmonicida. Smears of kidney material from yearling and adult trout were stained by Gram's method and examined for the presence of the kidney disease bacterium. Cultures from all trout were incubated at 20°-25° C., examined every other day for growth, and discarded after 6 days if growth did not occur. Cultures which showed growth were streaked on TSA to determine the type or types of bacteria present. A slide agglutination test, using rabbit anti-A. salmonicida antiserum, was run on any isolate suspected of being A. salmonicida.

Detailed results of the examinations are given in Table 1. We neither isolated A. salmonicida nor detected agglutinins in the sera or plasma of trouts at White Sulphur Springs NFH, so this hatchery was classified as negative for furunculosis. Trouts at Bowden NFH have been known to have furunculosis but the organism was isolated only from brook trout just after an outbreak. Adult rainbow trout at Bowden were probably carriers because they had agglutinins, but A. salmonicida was not isolated. Results obtained at the Cortland NFH showed that the yearling rainbow trout had agglutinins against A. salmonicida but, again, A. salmonicida was not isolated. This population must be considered as a possible carrier and all dead fish should be examined bacteriologically for the presence of A. salmonicida. Since furunculosis has not been reported at Cortland in the last 10 years, at least, and A. salmonicida was not isolated, the station can be considered as free from furunculosis from a practical standpoint. The rainbow populations should be closely watched.

Kidney disease bacteria were not seen in any stained kidney smears from trout at the three hatcheries, but a latent infection of kidney disease might easily be missed with this procedure.

The failure to isolate A. salmonicida, especially from the adult rainbow at Bowden which were probably furunculosis carriers, could be due to some inhibiting substance.

While our culture methods are adequate for detecting A. salmonicida in a population of salmonids that have just experienced an outbreak of furunculosis, they are not adequate for reliable detection of the bacterium in carriers. Detection of A. salmonicida in carriers is apparently difficult; recently Klontz showed by immunofluorescence that a wild population of apparently healthy suckers harbored A. salmonicida in the folds of their gut wall, but the organism could not be cultured. Furunculosis developed in these fish 7 to 10 days after thermal or physical stress. While immunofluorescent technique is promising for detecting the carrier state of furunculosis, it is not yet practical in the field. Since A. salmonicida is present in the gut of at least some carriers, the bacterium may be shed in the feces.

G. L. Bullock
Steve Leek
Ivan McElwain
L. L. Pettijohn
H. M. Stuckey
R. E. Putz

Table 1.--Results of furunculosis and kidney disease survey at three Federal hatcheries

Hatchery	Trout	Lot. No.	No. examined	Positive blood culture 1/	Positive kidney culture 1/	Presence of A. salmonicida	Presence of A. salmonicida agg. antibodies
Cortland	Yearling brook	8C	58	13/58 2/	0/10	Negative	Negative
	" rainbow	8C	57	6/57	2/12	Negative	19/57 and 33/42 3/
	" brown	8C	58	4/58	2/11	Negative	Negative
	Brown brood	66,7C	28	2/28	1/5	Negative	Negative
	Fingerling rainbow	9M	146	-	48/246	Negative	Negative
	" brook	9C	146	-	13/146	Negative	Negative
	" brown	9C	146	-	31/146	Negative	Negative
White Sulphur Springs	Large rainbow	8C	58	4/58	1/15	Negative	Negative
	" brook	7Y1	28	1/28	2/5	Negative	Negative
	Small rainbow	M	146	-	48/146	Negative	Negative
	" brook (Leetown)	9WS	146	-	5/146	Negative	Negative
	" brook (Owhi)	9WS	146	-	8/146	Negative	Negative
Bowden	2 and 3 year rainbow	8BNZ 7BNZ	58	3/58	2/58	Negative	39/58
	Yearling brook	8UWV	58	4/58	9/58	Positive (7 cultures from 5 trout - 3 blood, 4 kidney)	Negative

Microscopic examination for kidney disease organisms was negative at all three stations.

1/ other than A. salmonicida

2/ numerator = number of fish with bacteria or agglutinins; denominator = number of fish examined

3/ resample of same population

VIROLOGY AND CELL CULTURE

Channel catfish virus (CCV)

The work reported here was largely carried out on a training assignment at the Laboratory of Virology, St. Jude Children's Research Hospital, Memphis, Tenn.

Auburn strain CCV obtained from Dr. Nikola Fijan, was cloned by plaquing with standard gel overlay procedures and stocks designated clone A were grown at the Eastern Fish Disease Laboratory and preserved at -80° C. for use in the research at Memphis.

Two-phase (gel-liquid) procedures are used at St. Jude for plaque assay of polyhedral cytoplasmic DNA frog virus in fish and mammalian cells. These procedures were modified for plaquing CCV in the brown bullhead (BB) cell line. Excellent results were eventually obtained, and they showed a linear relation between virus dilution and plaque number (233, 21, 3) (Figure 1).

The BB cells did not grow at 33° C., and the quantity of virus replicated at 10° C. was only 6 to 8 times greater than input, so growth curves were plotted from data obtained at 25° and 30° C. At 30° C., CCV clone A had a lag phase of about three hours but new virus appeared by the fourth hour post-infection. Exponential growth of virus occurred for four hours, and growth began to level off at 10 hours. Maximum amounts of virus were attained by the 16th hour (Figure 2).

A parallel study by electron microscopy was carried out in collaboration with Dr. R. W. Darlington, staff electronmicroscopist. The work showed CCV to be synthesized in the nucleus and that it is an encapsulated icosahedron with well defined capsomeres apparently having a hollow configuration. Mean virion diameter of unencapsulated particles is about 125 nm, but resolution was not sufficient to determine the number of capsomeres. Sequential changes of infected cells as seen by electron microscopy have been documented, and a manuscript is in preparation.

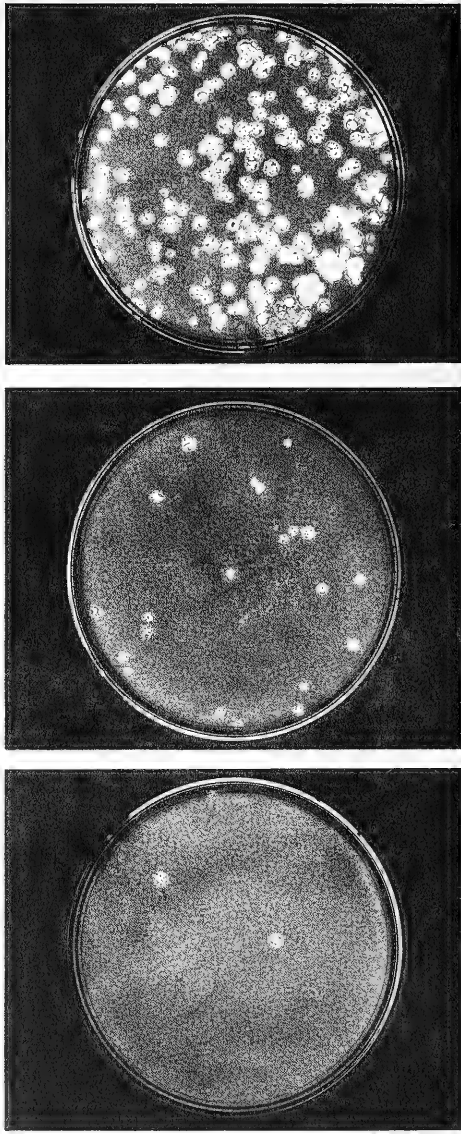


Figure 1.--Stained dish cultures of the brown bullhead (BB) cell line showing plaques produced by the channel catfish virus. Top, 10^{-4} dilution; middle, 10^{-5} dilution; bottom, 10^{-6} dilution.

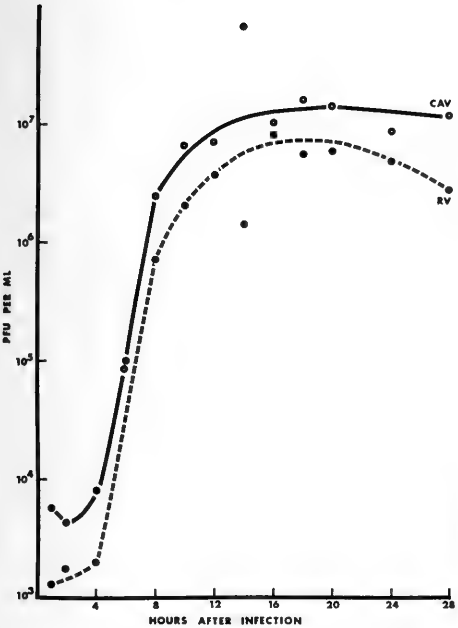


Figure 2.--Representative one-step growth curve of channel catfish virus in brown bullhead cells at 30° C. RV indicates released virus and CAV indicates that which remains cell-associated.

A similar study of sequential changes at 30° C. was carried out by light microscopy of infected BB cells. The essential changes were as follows: (cf. growth curve--Figure 2, Figure 3).

Hour 2 Light basophilia, beginning margination of chromatin and cell fusion.

Hour 4 Syncytia contain 3 to 5 nuclei. Beginning intranuclear inclusions.

Hour 6 Inclusions well-defined.

Hour 8 Increased basophilia, nuclear margins fading, syncytia with 6 to 10 nuclei.

Hour 10 Chromatin condensed internally and shifted away from nuclear margins. Six to 15 nuclei in largest syncytia. Some cells totally pyknotic and condensed, sloughing begins.

Hour 12 Syncytia increase in size to contain over 20 nuclei. Multiple intranuclear inclusions, some nuclei fragmenting within syncytia.

Hour 14 Nuclear dissolution continues. All cells in syncytia and/or pyknotic.

Hour 19 Lysis advanced and most cells sloughed.

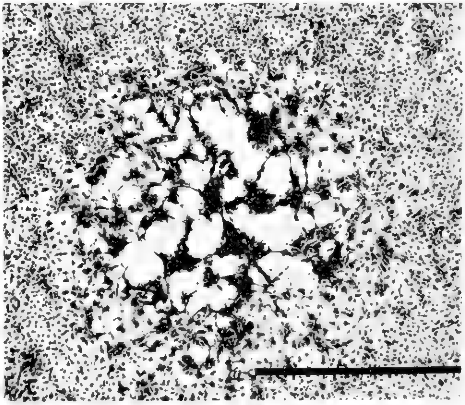


Figure 3.--Focal infection (a plaque) of channel catfish virus at 27 hours of 30° C. incubation. Terminal effects of the virus are evident as necrosis in the plaque center. Beyond the center, syncytia, so characteristic of this virus, are readily seen. The plaque perimeter contains pyknotic cells, the first visible change produced by the virus. Scale represents 1 mm.

CCV Clone A was found to be totally inactivated by extraction with ether or with chloroform. The virus was not replicated in AKRP (frog), primary chick embryo, BHK (hamster), Hela (human), H.Ep-2 (human), WI38 (human), primary African green monkey, Rhesus monkey, rabbit kidney and human embryonic kidney cell cultures. All the lines tested support growth of one or more Herpes viruses from other animals.

Nuclear replication and envelopment by nuclear or cytoplasmic membranes, a size of about 100 to 125 nm, and icosahedral morphology with hollow capsomeres indicate that CCV is a member of the Herpes virus group. Extreme sensitivity to lipid solvents, an unusually great host and host cell specificity and the induction of syncytium formation are all characteristics which support placement in the Herpes virus group. Determination of nucleic acid type remains to be done.

Experimental infections have been attempted with fingerling channel catfish (Ictalurus punctatus) at the Fish Farming Experimental Station, Stuttgart, Ark., but the fish have succumbed to virus only after massive injection and when the temperature was 30° C. and over. Additional trials are in progress to learn the histopathology of this disease, to implement a search for virus in carriers.

Needle biopsy of catfish kidney tissue was attempted in an effort to find a non-lethal way of virologically assaying adult tissues. The efforts were not successful and failure was attributed to the soft texture of internal organ tissue. Skeletal muscle could be successfully sampled with several different biopsy needles.

Periodic assay of CCV preparations indicates that retention of infectivity at 4° C. is good if cell culture harvests are in the medium with 10 percent serum levels. Infectivity is not maintained as well in lower serum levels.

Cold-blooded animal cell and tissue culture

RTG-2 and RTF-1 cells, the first and oldest established lines of fish cells, have now been carried in continuous cultivation for 10 years. The former cell line is still the culture requested most frequently from us--in 1969, 13 of 18 requests which we felt could not be referred to commercial sources or to the American Type Culture Collection were for RTG-2 cells. The RTG-2 is also employed in the bioprotocol for testing lunar soil.

Dr. Tokuo Sano, visiting scientist from the University of Tokyo, established primary monolayer cultures from eels (Anguilla rostrata) in preparation for return to his own laboratory and virological work with A. japonica. The cultures in general grow slowly and subcultures are established only with prolonged incubation. The best nevertheless persist.

Several tissues from spent adult lampreys (Petromyzon marinus) were trypsinized for preparation of monolayer cell cultures. Ten different media were tested but none proved better than that which we presently use for larval lamprey tissue. Thus far, cultures are maintained best at 15° C. or lower. Cells from adult tissues still show metabolic activity after 9 months in vitro whereas explants of ammocoete heart are still beating after 17 months in culture.

It is generally recognized that for detection and assay of virus, plaquing is more sensitive and accurate than endpoint of cytopathology determined in cultures grown in liquid medium.

Because of pH control and other considerations, plaquing is usually carried out in sealed vessels or in petri dish-type cultures in partial CO_2 atmospheres; both methods have disadvantages, and a compromise would use dish-type cultures in normal atmosphere.

The brown bullhead (BB) cell line used for the channel catfish virus (CCV) research described elsewhere in this report has been grown in Eagle's Minimal Essential Medium (MEM) containing a bicarbonate buffer which provides pH control at equilibration with CO_2 in sealed vessels or in CO_2 incubators. Leibovitz' Medium L-15 was designed to maintain a physiological pH at normal atmosphere, but in spite of repeated attempts, the BB cell line could not be adapted to growth in Medium L-15. Thus far, there has been no report of fish cell culture and virus plaquing in dish-type cultures incubated in normal atmosphere.

Several different buffer systems were compared for their ability to provide pH control in Eagle's MEM and ultimately for that medium to sustain BB cell growth and in turn efficiency of plaquing CCV. Tris (hydroxymethyl) aminomethane and N-2 hydroxy-ethylipiperazine N'-2-ethanesulfonic acid buffers both provided good pH control in BB cultures grown in normal atmosphere; moreover, results with the latter equaled or surpassed those obtained with control cultures grown in regular MEM (Table 2).

From this work, fish cell culture techniques have been advanced and a more accurate and sensitive virological assay system has resulted.

Ken Wolf and M. C. Quimby

PARASITOLOGY

Experimental transmission of Myxosoma cerebralis (whirling disease) and effect of freezing on the spores

Before critical research can be done on treatment and control methods, whirling disease must be reliably reproduced under experimental conditions. Although young salmonids become easily infected when placed in contaminated waters, it is very difficult to infect such fish at will in the laboratory. To find a reliable method of reproducing the disease, spores in infected fish tissues were introduced to 24 aquaria, 16 of which contained 3 to 5 inches of mud taken from warmwater fish ponds. Some of the aquaria were 150-liter stainless steel tanks, some were 75-liter glass aquaria, and some were 340-liter fiberglass tanks. Spring water (12° C.) flowing at about 600 ml/min was supplied during "aging" of the spores. After

Table 2.--Comparison of BB cell cultures grown in Eagle's Minimal Essential Medium with various buffer systems

Buffer	Relative culture growth rate	pH control	Relative efficiency of plaquing
Sodium bicarbonate (Control)	100%	Excellent	10.4×10^6 pfu/ml
Sodium-potassium phosphates	35 to 55%	Fair	10.3×10^6 pfu/ml
Tris(hydroxymethyl) amino methane	66 to 88%	Very good	13.6×10^6 pfu/ml
N-2 hydroxyethyl piperazine-N'-2-ethanesulfonic acid	92 to 113%	Excellent	12.0×10^6 pfu/ml

[1] In suitable replicates, various stocks of channel catfish virus were plaqued on cells grown in the several modifications of medium Representative values are given in plaque forming units (pfu).

Similarly modified media were used for dish-type culture of RTG-2 cells, and it was found that the basic methodology will permit plaquing of Infectious Pancreatic Necrosis, Egtved, Infectious Hematopoietic Necrosis ai Chinook Salmon viruses in standard incubators.

the fish were added, the flow was increased to about 1800 ml/min. All aquaria had standpipe drains to facilitate the retention of spores. Fifty to 100 rainbow trout fry, usually 3-4 weeks old, but 10 weeks old in 2 aquaria, were stocked at regular intervals from 0 to 6 months after the spores were added. The fish were observed and were autopsied and examined for spores 4 to 6 months after stocking.

Fish became infected in 7 of the 24 aquaria. Only those aquaria containing mud and spores "aged" 3.5 to 6 months contained infected fish (Table 3). Symptoms were first noticed 2.5 to 3.5 months after the fry were placed in the aquaria. In 5 aquaria the symptoms were typical and spores were numerous in the fish. However, in 2 aquaria no symptoms were noticed but examination of cartilage showed spores in small numbers.

of the spores. In some of the experiments infected trout heads were frozen at -20° C. for 2 months, and then cut up and introduced to aquaria. Freezing did not kill the spores (Table 4).

Experiments 67-6(3) is being maintained to see how long a contaminated facility remains so. Spores were placed in the aquarium on August 9, 1967. After fry were added on November 28, 1967, the mud surface was stirred gently several times at weekly intervals. Symptoms were seen February 16, 1968, and immature spores were present. These fish were removed and new fry added on February 23; the second lot became infected and were removed May 21. This procedure was repeated 9 more times, with the fish becoming infected. The first 3 batches of fish were left in the aquarium about 3 months (long enough for spore production), but the last 8 were removed before production. Dead fish were removed promptly during this experiment. The results demonstrate that the spores remain viable for

Table 3.--Effect of "aging" on _Myxosoma cerebralis_ spores

Experiment No.	Facility	3-5" of mud added or not	Spores "aged" in aquaria, months	Age of fry at start, weeks	Presence of spores in fish
66-1B	150-liter steel tank	no	4	3	0
66-1A	" "	yes	4	3	+
68-14	" "	no	2	3	0
67-6(1)	340-liter fiberglass	yes	3	10	+
67-6(3)	" "	yes	3.5	3	+
67-6(4)	" "	yes	3.5	3	+
68-11	" "	yes	6	3	0
68-13	" "	no	2.5	3	0
68-14	150-liter steel tank	no	2.5	3	0
69-5A	75-liter aquarium	no	0	10	0
69-6	" "	no	3.5	3	0
69-7	" "	no	3.5	3	0
69-8	" "	no	3.5	3	0

From these experiments we assume that some mud is necessary to produce infection. The spores became infective 3 to 6 months after placing them in the aquaria, but we do not know the minimum and maximum infective ages

Table 4.--Effect of freezing and "aging" on Myxosoma cerebralis spores

Experiment No.	Facility	3-5" of mud added or not	Spores frozen -20°C, days	Spores "aged" in aquaria, months	Age of fry at start, weeks	Presence of spores in fish
66-16A	150-liter steel tank	yes	270	4.5	4	0
67-1B	" "	yes	18	4	10	+
67-6(2)	340-liter fiberglass	yes	3	0	10	0
67-6(5)	" "	yes	3	3.5	10	0
67-6(6)	" "	yes	60	2.5	10	0
68-5	" "	yes	60	6	3	+
68-6	" "	yes	60	6	3	0
68-7	" "	yes	60	6	3	+
68-8	" "	yes	330	6	3	0
68-9	" "	yes	330	6	3	0
68-10	" "	yes	330	6	3	0
69-1	" "	no	480	4.5	3	0
69-2	" "	no	960	5.5	3	0

22 months or the live infected fish shed infective units, or both.

The actual mode of transmission of M. cerebralis has never been experimentally determined; therefore a filtration experiment was initiated to pinpoint the size range of the infective unit. Water and sediment from an infected tank, where M. cerebralis has been maintained since the first quarter of 1968, were siphoned into a 20-liter container and from this filtered through filters of 14 μ to 1.2 μ potasity. This was done each day for 5 consecutive days. After running the water and sediment through each size filter, the filters with residue were placed in glass aquaria with susceptible trout. Results are shown in Table 5. Presence of spores of M. cerebralis in infected trout was confirmed by histopathological examination. This experiment is being run again with smaller trout and more consecutive days of filtering. The spores are actually 7.5 to 9.5 μ in diameter. The infective units, whether present as free spores or carried by larger organisms, were retained by the 12 μ filter. It may be that free spores were adsorbed to the filter pad or trapped in debris.

R. E. Putz

Serodiagnosis of whirling disease

At present whirling disease of salmonids cannot be detected in asymptomatic carrier fish without sacrificing the fish, and it is sometimes very difficult to find spores in adult trout. Therefore, an experiment was initiated to try to diagnose the disease using a modified indirect fluorescent antibody technique. Rainbow trout globulin (to be used as an antigen) was prepared by fractionating the trout serum, and was injected into rabbits. The system was then tested .

A positive control system, using the indirect fluorescent antibody technique developed for salmonicida was run, as well as a negative control system using trout serum from non-myxosoma-infected trout. So far the experiments have not given satisfactory results because those not exposed to fluorescent antibody fluoresced as strongly as controls. Untreated spores of M. cerebralis fluoresced in ultraviolet light.

R. E. Putz and Ivan McElwain, trainee

Table 5.--Results of *Myxosoma* filtration experiment

	Control 1. No. addition of H_2O and sediment from infected tank	Control 2. Addition of H_2O and sediment from infected tank. Not filtered	Tank with 14μ filter pad and residue	Tank with 8μ filter pad and residue	Tank with 3μ filter pad and residue	Tank with 1.2μ filter pad and residue
Trout 3 months after last filtration	Negative for spores	Positive for spores	Positive for spores	Negative for spores	Negative for spores	Negative for spores

Effects of disinfecting agents and other chemicals on spores of Myxosoma cerebralis

The effects of calcium hydroxide, potassium hydroxide, calcium chloride, calcium hypochlorite, ammonium chloride, and sodium borate on Myxosoma spores are being studied by a local student, Lyle Hoffman. So far, a high concentration of calcium oxide and potassium hydroxide at 0.5 percent has consistently destroyed the spores.

Bird transmission of Myxosoma cerebralis

Normal Myxosoma spores are carried in the feces of kingfishers and herons which have fed on infected fish. Mr. T. Udell Myers, Castalia, Ohio, fed infected fish to captive great blue herons, collected the spore-bearing feces, and shipped it to Leetown. We have seeded experimental tanks with it, allowed the spores to "age," and have added swim-up rainbow trout fry to see if the spores are infective.

Isolation and morphology of M. cerebralis spores

Preliminary work has been started with pepsin HCl digest of infected trout heads. Following digest, the material was strained through 224-, 154-, and 70-micron mesh screens. Fairly good concentrations of spores were recovered and sent to Dr. J. Lom for electron photomicrography. Spores from other species of salmonids will also be collected and a revised description of the spore prepared.

HISTOPATHOLOGY

An epizootic involving a fungus infection in the kidney and other viscera of adult rainbow trout was referred by Jimmy Camper, Hatchery Biologist, Region 4. There were significant mortalities, and gross examination revealed swollen kidneys with dark brown spots of about 2 mm. In severely infected fish the brown spots were seen elsewhere in the viscera. Examination of wet tissue squashes and histological sections of kidney showed that the fungus had branched mycelium and hyphae ranging from 1 to 2.5 μ in diameter (Figure 4). No spores were observed. The Western Fish Disease Laboratory will further investigate this fungus.

G. L. Hoffman and C. E. Dunbar (deceased)

Figure 4.--Undescribed fungus from the kidney of adult rainbow trout.

CHEMOTHERAPY OF FISH DISEASES

During the latter part of the year, we cooperated with the Hoffman-LaRoche Company to evaluate sulfisoxazole, sulfadimethoxine, and a combination of sulfadimethoxine and pyrimidine for treatment of bacterial fish diseases.

Efficacy tests were delayed because of a low water supply and difficulty in establishing infections which were not fulminating.

The results of force-feeding trials indicate that doses of over two grams of sulfisoxazole per kilogram of fish weight are non-toxic to rainbow trout. Free-choice feeding of the drugs clearly indicated that the combination drug had a low palatibility to rainbow trout. At the highest dose given, the fish refused to eat after one feeding. Table 6 gives the results of this experiment as measured by weight gain or loss.

R. L. Hoffman and G. L. Bullock

Table 6. Effects of free-choice feeding of drugs on weight changes in rainbow trout at 12.5°C.

Days	Drug	Dose mg/kg	Weight (gm) Gain	Loss
12	Sulfisoxazole	220	+ 140	
		440	+ 134	
		880	+ 55	
	Sulfadimethoxine	220	+ 129	
		440	+ 46	
		880		- 54
	R05-0037	220		- 124
		440		- 51
		880		- 30
	Control	0	+ 130	
		0	+ 122	
		0	+ 132	
10	R05-0037	100	Terminated--fish not feeding	
		50	+ 191	
	Control	0	+ 217	

NEOPLASTIC FISH DISEASES

A report on Mr. C. E. Dunbar's work on the etiology of visceral granuloma is being prepared for publication. This disease is definitely diet-associated, and cottonseed meal is an important factor. When cottonseed meal was eliminated from a meat-meal diet known to produce the disease, visceral granuloma incidence was very low (4.7 percent vs. 91.3 percent). Cottonseed meal added to a synthetic, non-granuloma-producing diet caused an incidence of 21 percent. Gossypol is not the principal cause of this high incidence; when added to the synthetic diet at a rate of 333 ppm, it produced an incidence of only 2.6 percent.

It is currently speculated that dietary mineral imbalance (e.g., calcium, phosphorus, and/or magnesium) occurs, resulting in pathological deposition of calcium and osmoregulatory difficulties.

R. L. Herman

GENERAL

Diagnostic services

R. L. Herman assumed the duties of histopathologist following the death of Mr. C. E. Dunbar.

Reference tissues from *Anguilla rostrata*, *Petromyzon marinus*, and *Acipenser* sp. were added to the laboratory collection during the year. Excellent specimens of rainbow trout infected with infectious hematopoietic necrosis (IHN) became available through routine diagnostic examination.

Dr. Fred Meyer, Fish Farming Experimental Station, Stuttgart, Ark., sent several isolates of a bacterium causing death among cultured catfish. The organism is not one of the common catfish pathogens; it appears to belong in the enteric group. Fingerling catfish injected with the organism or exposed to a heavy cell suspension became diseased and died two days after injection and five days after exposure. External pathology with experimental infections included hemorrhage around the mouth, at the base of fins, and in the visceral mass. Microscopic examination of tissues of infected catfish showed only focal necrosis in the pancreas.

G. L. Bullock, H. M. Stuckey and R. L. Herman

More than 35 specimens of parasites were received for diagnosis, most notable of which were: unknown granulomas in goldfish viscera; microsporida in catfish heart and intestinal wall; microsporida in starry flounder from California; *Posthodiplostomum minimum* from the eye and ovary of *Gambusia affinis*; *Contracaecum* and *Proteocephalus* larvae from an emaciated 12 oz. largemount bass 16 inches long; and an *Ichthyophonus*-like parasite from frog muscle.

Noteworthy and encouraging from the point of international fish disease control was a request to examine and certify bluegill fry to be free of disease before shipping to Iran by Eugene Surber, Virginia Commission of Game and Inland Fisheries.

G. L. Hoffman and R. E. Putz

Training and committees

Messrs. Bullock and Putz, and former trainee Charles Berry, Water Pollution Board, State of Virginia, spent the month of July at the Lewis Calder Conservation Study Center, Armonk, N. Y. They were studying parasites, bacterial flora, and several blood parameters of lake fish as part of ecological study of a lake sponsored by the Biology Department at Fordham University.

Dr. Glenn Hoffman serves on the council of the Wildlife Disease Association and its Awards Committee; he is also on the International Committee and Awards Committee of the American Society of Parasitologists and the Fish Disease Committee of the American Fisheries Society.

A two-day workshop on general biology and diagnosis of whirling disease (*M. cerebralis*) was conducted at Leetown at the request of the U. S. Trout Farmers Association on February 20 and 21. This was the first project arranged by the Association's Disease Research Committee, and was organized by T. Udell Meyers, Biologist, Castalia Farms, Ohio. Sixteen trout farmers and biologists attended. Dr. Hoffman participated in a similar course at the Western Fish Disease Laboratory, August 14 and 15.

Florence T. Wright, Librarian, attended a Departmental Library Workshop held in Denver, Colo. on September 29, and a December 6 Board Meeting of the West Virginia Library Association as Chairman of the Special Library section.

WESTERN FISH DISEASE LABORATORY

Seattle, Washington
Robert R. Rucker, Director

HIGHLIGHTS

Furanace may prove satisfactory for separating the two genera Pseudomonas and Aeromonas.

Adult rainbow trout are Infectious Hematopoietic Necrosis "carriers," and techniques for identifying "carrier" fish have been developed.

Virions of Infectious Hematopoietic Necrosis were observed with an electron microscope in various stages of development in fish tissues.

Albino rainbow trout exhibited an anamnestic or memory response to the secondary inoculation of an antigen. The lag time between inoculation and antibody rise was considerably reduced when a previous stimulation had been given.

Immunoelectrophoresis of the sonicated supernatant of Aeromonas salmonicida displayed some of the antigens which will induce antibody formation when inoculated into rabbits and rainbow trout.

Cytophaga psychrophila has been found to be closely correlated with the occurrence of whirling disease of coho salmon.

Field experiments indicate that Furanace (P-7138) is effective in the control of myxobacterial infections.

For yearling rainbow trout, benzocaine or neutralized MS-222 rather than MS-222 (acid) are the anesthetics of choice for clinical chemistry measurements unless anesthesia is limited to 3-5 minutes.

Blood cholesterol and urea levels of rainbow trout fall to about half normal value in a few days when rainbow trout are subjected to crowding stress. The values slowly return to normal.

The estimated normal clinical chemistry ranges for 7 chemical components from blood and interrenal tissue have been determined for yearling rainbow trout.

BACTERIOLOGY

Isolation and identification of organisms associated with fish diseases

Rainbow trout from several epizootics of unknown causes were tested for mycoplasma. None were isolated.

Bacterial cultures were obtained from rainbow trout at a private hatchery in Washington and from chinook salmon at a Washington State Department of Fisheries hatchery. These isolates were very similar to the R. M. bacterium usually associated with rainbow trout from Idaho. The isolates from trout differed in that they were non-motile in contrast to the motile R. M. organisms normally seen; however, they conformed in all other respects to R. M. bacterium.

Preliminary investigations were started to determine the taxonomic value of extracellular deoxyribonuclease production from bacteria pathogenic to fish. Three cultures each of

Pseudomonas sp., A. salmonicida, R. M. bacterium, A. liquefaciens, A. hydrophila, A. punctata, and Vibrio sp. were grown on a culture medium containing deoxyribonucleic acid (DNA). A chemical test was used to demonstrate the occurrence of DNA degradation. Degradation occurred in all cultures except the Pseudomonas sp. and the R. M. bacterium. Cytophaga psychrophila and Chondrococcus columnaris did not grow on the test medium.

An unidentified fungal disease of rainbow trout from a Tennessee hatchery is being studied. We are unable to infect fish after repeated feeding of fungal cultures; however, death occurred rapidly following intraperitoneal injection. Tissue reaction to the fungus is similar to that seen in fish infected with Ichythosporidium. In the original material, cells similar to giant-cells and granulomatous areas were observed in abundance (Figure 1). A similar species has been isolated on several occasions from our stock coho salmon. We are attempting to identify the fungus (Figure 2).

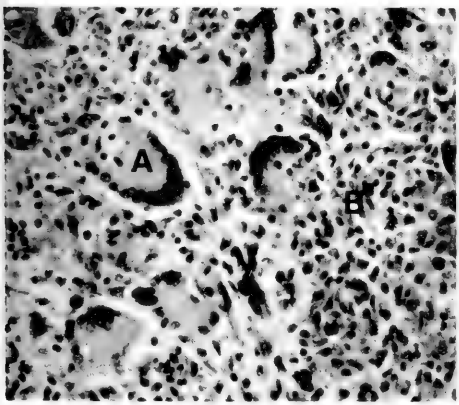


Figure 1.--Mid-kidney of fungus-infected rainbow trout showing cells similar to giant-cells (A) and granulomatous area (B). X320 Giemsa stain.

Myxobacteria

Several myxobacterial cultures have been frozen and held at -40 C. in order to determine length of survival. Hopefully this procedure can be used for maintaining a stock culture collection. The cultures were grown in a semi-solid agar medium prior to being frozen. Two cultures of Cytophaga psychrophila were viable after 10 months in the freezer and the Chondrococcus columnaris culture was viable after 6 months.

Figure 2.--External lesion on a coho salmon infected with fungus.

Toxins

It has been speculated that toxins may be involved in the pathogenesis of the disease caused by Chondrococcus columnaris. We were unable to demonstrate endotoxins, so diverted our efforts towards the isolation of exotoxins and proteolytic enzymes. The preparations we have obtained do not elicit a response when injected into fish. Proteolytic enzymes produced by some bacteria can destroy the exotoxin produced by the same organism. We are currently attempting to culture strains of C. columnaris in the presence of proteolytic enzyme inhibitors, thus protecting the exotoxin if one is being produced.

Therapeutics

Anti-germ 50, a quaternary ammonium compound, was tested against Chondrococcus columnaris at concentrations ranging from 1:5000 through 1:100,000. Inhibition was obtained at the levels tested.

Spectam, an experimental antibiotic, was tested against 6 strains of myxobacteria from fish with cold-water disease in Washington and Oregon hatcheries. There was no inhibition at a drug concentration of 12.5 micrograms/ml. These 6 strains were also tested against Furanace at concentrations varying from 0.048 micrograms/ml to 12.5 micrograms/ml. One-week post-inoculation growth was observed only in the control tubes containing no drug.

Ten strains each of Aeromonas salmonicida, A. liquefaciens, Red Mouth (R. M.) bacterium, Pseudomonas sp. and Vibrio sp. were tested against Furanace at concentrations ranging from 0.195 micrograms/ml to 12.5 micrograms/ml. The Pseudomonas strains were the most resistant and the strains of A. salmonicida were most sensitive. None of the 10 Pseudomonas cultures were inhibited by 12.5 micrograms/ml and only 2 of the 10 A. salmonicida cultures were able to grow at a concentration of 0.78 micrograms/ml. Of the 10 A. liquefaciens cultures only one grew at a concentration of 3.125 micrograms/ml. Further study may show that sensitivity to Furanace might be of value in separating members of the genera Pseudomonas and Aeromonas. Six of the Vibrio cultures were not inhibited by 12.5 micrograms/ml while all of the R. M. bacterium strains failed to grow at a concentration of 6.25 micrograms/ml.

A. J. Ross

VIROLOGY

Infectious Hematopoietic Necrosis (IHN)

IHN virus was originally isolated from juvenile rainbow trout and sockeye salmon in British Columbia in 1967. The disease recurred in sockeye salmon in 1968. Between 100 and 175 adult fish from 5 races of sockeye salmon were examined for IHN virus in early 1969, to determine geographic distribution of the virus in the Fraser River drainage and whether adult sockeye salmon were "carriers" of the disease. Filtered homogenates from kidney and intestinal material were inoculated onto FHM cells. No virus was isolated from any race of fish. Subsequently, the disease did not occur in the 1969 juvenile sockeye salmon and the disease has not been diagnosed in Canadian rainbow trout since 1967.

In 1969 we confirmed the diagnosis of IHN in rainbow trout from 5 new localities in 4 states. The mortality rates ranged from 5 to 98 percent and were found in fish from 3 to 5 weeks post hatching up to 8 months of age. In all cases to date epizootics occurred at water temperatures below 50° F. and the disease incidence appeared to decrease with increase in age (size) and water temperature. In one case the disease was controlled by elevating the water temperature to 60° F. We have confirmed the temperature correlation experimentally.

Methods of disease transmission and detection of "carrier" fish are being investigated. The disease can be transmitted by injection and from fish to fish, and transovarian transmission and exposure via feed are also being tested. We have demonstrated the virus on tissue culture from the ovarian fluid, pyloric caeca, and intestine of carrier adult fish, but not from the blood, kidney, or liver from the same fish. The highest titers of virus occurred in the pyloric caeca. We found that for routine sampling of brood stock, five-fish pools of ovarian fluid will suffice for detecting carriers. It was also found that fathead minnow (FHM) tissue cultures are more sensitive than rainbow trout gonad (RTG-2) cells in detecting carrier fish (Figure 3).

Since increased water temperature controlled IHN disease in juvenile fish, adult trout prior to spawning were placed in 56 to 58° F. water for 9-12 days, and then returned to 48-50° F. water to determine if the elevated water temperature would clear the fish of the virus. Fish kept at 50° F. throughout the experiment had a minimum carrier incidence of 3.5 percent in a population of 170 fish. Immediately following the temperature treatment, no carrier fish could be detected in 158 fish. However, within two weeks after being resubjected to 50° F., 81 fish had a minimum carrier rate of 18.5 percent. Because eggs were poor if fish were spawned within one week after being removed from the high temperature and because the virus recurred within two weeks, this method is not a practical way of eliminating the disease.

Attempts were made to induce an antibody response in rabbits, using techniques similar to

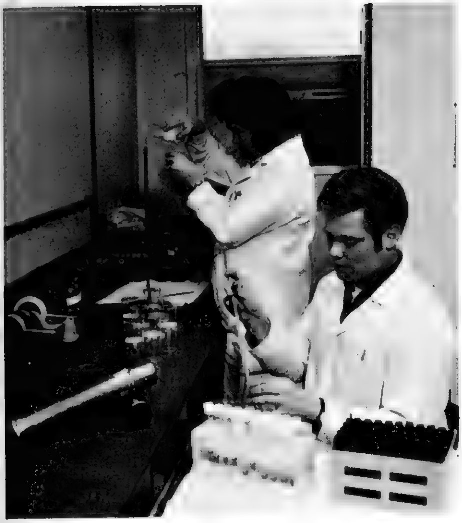


Figure 3.--Fish specimens being processed for virus isolation. Betty Jefferson, Lummi Indian aquaculture trainee, is preparing bacteria-free filtrates for John Pietsch, Washington State Department of Fisheries Cooperator, to inoculate onto fish tissue cultures.

those developed for "Egtved" virus. Rabbits were injected with purified and unpurified FHM tissue culture-grown IHN virus with and without the use of adjuvants, once a week for 3 months. No IHN-neutralizing antibodies were detected from any preparation. In addition, serum from carrier trout showed no neutralizing antibodies against IHN virus isolated from the same fish. We plan to repeat the experiments using concentrated virus preparation.

A previous electron microscope study of IHN virus from FHM cells showed numerous bullet-shaped virus particles. To determine if similar structures could be found in diseased fish, fingerling sockeye salmon were infected with stock strain IHN-4. After 10 days, kidney, liver, spleen, pyloric caeca, and liver from 2 moribund fish were fixed with osmium tetroxide and examined with an electron microscope. Necrosis was seen in all tissues, confirming the observations previously reported using the light microscope, but virions of IHN were seen only in the kidney, spleen, and pyloric caeca. Virions appeared to be clustered around certain cells in the kidney and spleen, but because of the extensive necrosis the cell type could not be identified. Compared to normal tissues the lymphocytes and cells of the early erythrocytic series were absent. Mature erythrocytes appeared normal. In the pancreatic area virions were observed only in epithelial layers of what appeared to be the acinar ducts. Various stages of development were observed and mature virions measured 90 mμ wide by 158 mμ long. This agrees favorably with the measurements of virus particles found in tissue culture.

Donald F. Amend

IMMUNOPATHOLOGY

Anamnestic response

New techniques in immunology have provided means for better defining the immune response in salmonid fishes. For example, current methods of electrophoresis, immunoelectrophoresis, and column chromatography have helped in classifying and defining the nature of rainbow trout antibody.

Thirty albino rainbow trout were stimulated to produce specific antibody by one subcutaneous inoculation of Aeromonas salmonicida antigen. A sonicated, alum-precipitated, supernatant preparation of the antigen was used because it was previously found that this procedure enhances the immunogenicity. Weekly samples of one milliliter of blood were taken by heart puncture (Figure 4) and analyzed for antibody characteristics and titer. In this experiment a maximum titer was reached 5-8 weeks following the initial inoculation. A second stimulation was given 4 months after the primary inoculation.

Secondary inoculation resulted in the expected booster effect usually found in mammalian systems. This anamnestic or memory response was readily apparent one week after secondary inoculation. Serums from the primary and secondary responses were tested by immunoelectrophoresis against the inoculated antigen. Only one precipitin line occurred in

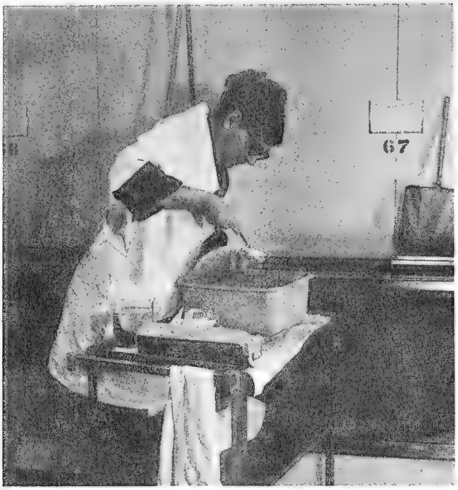


Figure 4.--Lummi Indian aquaculture trainee, Clarissa Finkbonner, obtaining blood from albino rainbow trout by heart puncture. The trout are being used for furunculosis (A. salmonicida) antibody formation studies.

Figure 5.--Sephadex chromatography columns are kept in a cold incubator. Serum samples are separated by filtration through the gel column; fractions are collected and analyzed for specific antibody content.

both cases. This line, however, was slightly skewed to the cathode. Upon further investigation it might be found that the antibody molecule is not a complete homogeneous entity--variations may exist. The same serums were run through a Sephadex G-200 gel column to further detect differences in antibody weights and configurations (Figures 5 and 6).

Vaccine study

The production of effective vaccines depends on a thorough knowledge of the antibody reaction in fish and especially the identification of the protective antibody. Aeromonas salmonicida cells were ruptured by sonication, and supernatant fluids were inoculated into rabbits and rainbow trout on regular schedules to determine which bacterial antigens would elicit a response. Gel immunoelectrophoresis was the method used for detecting the various antigens.

The rabbits produced humoral antibody to at least 14 antigens in the heterogeneous mixture which was inoculated. Antiserums from rainbow trout precipitated only those few antigens with slow anodic mobility (Figure 7).

These results do not necessarily demonstrate rainbow trout are less immunocompetent than rabbits, for it is known that different animals will react differently to various antigens. However, by these methods, we can begin to determine which antigens are of primary importance in giving rise to protective antibody in the trout.

D. P. Anderson

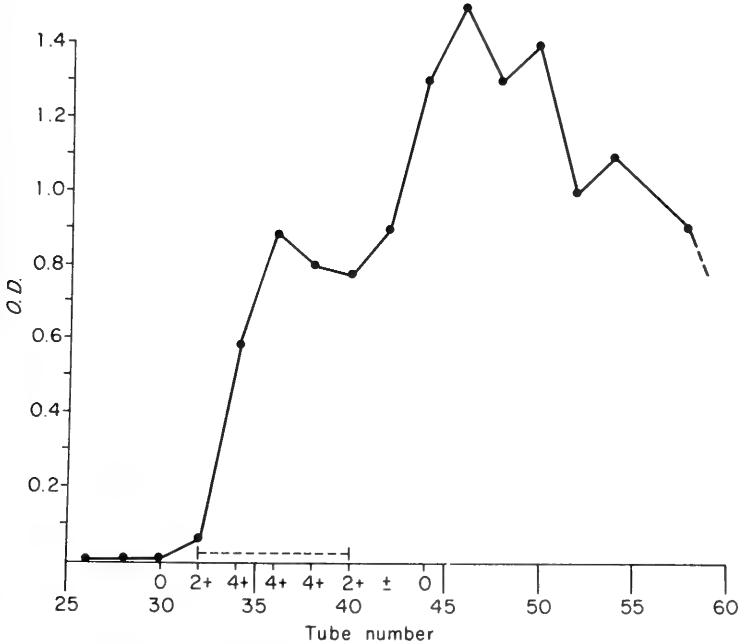


Figure 6.--The trout anti-A. salmonicida serum was fractionated by filtration through Sephadex G-200. The optical density (O.D.) is a measure of the amount of protein collected in the individual tube samples. Note that the agglutinating antibody as shown by dotted line occurred in tubes 32-40. Since the heaviest molecules are collected in the low-numbered tubes, this demonstrates that the antibody is a heavy component of the serum.

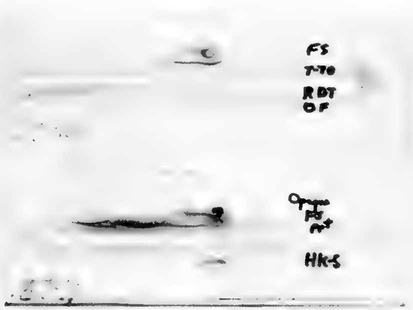


Figure 7.--The soluble antigens of A. salmonicida were placed in the top wells of both slides. After electrophoresis, the trough of the top slide was filled with anti-A. salmonicida trout serum and that of the bottom slide with anti-A. salmonicida rabbit serum. Note that the rabbit serum recognized many more of the antigens than the trout serum.

HISTOPATHOLOGY

Kidney disease

Examination of materials from a kidney disease histopathogenesis study was completed. Bacteriological and histopathological analyses were done on sockeye salmon fingerlings. Weekly samples of 5 fish were taken from each of three groups: A. Control--inoculated intraperitoneally with saline. B. Test-inoculated--inoculated intraperitoneally with kidney disease bacterium, Cornyebacterium sp. C. Test-fed--fed viable organisms which had been incorporated into the complete test diet.

Kidney tissue smears were made from all samples and gram stained to verify infection.

Histopathological tissue changes in the B group were observed in the 1st week samples. Hematopoietic hyperactivity was evident in the kidney with the presence of an increased number of immature cells ("stem" or "blast" cells) and with a decreased number of lymphocytes (Figures 8 and 9). Minute foci of macrophages containing bacteria were also seen. Another evidence of infection was the noticeable increase of leukocytes in the vascular system (Figure 10). By the 4th week the infection in the B group had become systemic. Sampling was discontinued the 6th week since there was just one surviving fish.

In the C (Test-fed) group microscopically observable tissue changes did not occur until the 5th week. Very small foci of macrophages in the kidney hematopoietic tissue were the earliest histopathological changes seen. Immature cells in the kidney and leukocytes were not as abundant as in the test-inoculated fish.

Of the specimens taken at the termination of the experiment (12th week), 2 of the 5 showed clinically observable pathological changes in the kidney. The septicemic and systemic nature of the infection was quite evident histologically. Bacteria-engorged macrophages (Figure 11) were present throughout the circulatory system and most of the other tissues.

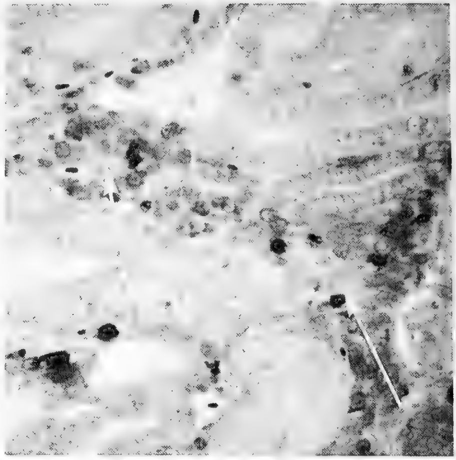


Figure 8.--Posterior kidney of control fingerling sockeye salmon showing hematopoietic area (arrows) and tubules. X900. Giemsa stain.

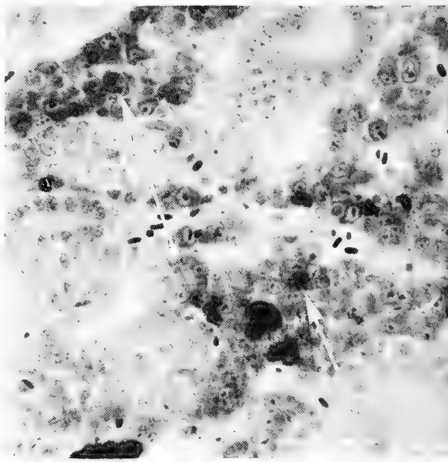


Figure 9.--Posterior kidney of 1-week test-inoculated fingerling sockeye salmon showing increasing number of immature "stem" cells in the hematopoietic area (arrows). X900. Giemsa stain.

Figure 10.--Heart tissue of 1-week test-inoculated fingerling sockeye salmon showing noticeable increase of leukocytes. X900. Giemsa stain.

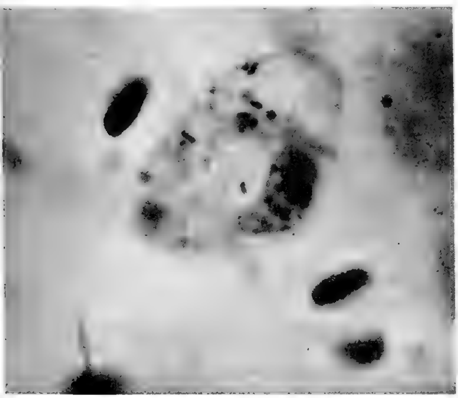


Figure 11.--Macrophage with phagocytized bacteria in an artery of 11th week test-fed fingerling sockeye salmon. X2800. Giemsa stain.

Whirling disease of coho salmon

Whirling disease of coho salmon has been prevalent in coho hatcheries. Clinically there are some similarities to whirling disease of trout, but etiologically and histologically these two diseases are quite different. High mortality has rarely been associated with the coho disease. In a given population only a very small percentage of coho become affected. Whirling or corkscrewing along the long axis of the body and scoliosis and lordosis are frequently observed in these fish.

Histologically, the specimens exhibiting the whirling symptom frequently exhibited massive tissue changes in the posterior portion of the skull, particularly in the area adjacent to the medulla oblongata. The vertebral column just posterior to the head was also often involved. Invasiveness of pathological tissue suggested a possible neoplasm. In the earlier specimens received at this laboratory no organisms were found in the lesions.

The specimens with spinal curvatures showed histopathological changes in the area of the curvature similar to those mentioned above. Here again, the invasiveness of the pathological tissues was quite evident.

During the last few years we received many coho salmon specimens showing almost identical clinical manifestations and histopathological changes. However, there was one atypical aspect; a myxobacterium, Cytophaga psychrophila, etiologic agent of cold water disease, was found in the majority of the lesions of these fish. This disease has been reported occasionally in chinook and sockeye salmon, but extensive pathological changes have not been present when it is found in these two species.

We are attempting to determine whether there is any relationship between the bacterium and the histopathological changes.

Diagnostics

Diagnostic materials (82 cases) for histopathological analyses were received from private, state, Federal agencies, and several foreign countries, including Canada, Chile, Scotland, and Sweden. Numerous samples this year were sent to us as IHN-suspect material. Several of these were found to be IHN-negative but had diffuse hepatic parenchymal cell degeneration. This year we have received increasing numbers of samples showing these hepatic cell changes. Although the etiological aspect of this condition is still not clear, the histopathological picture suggests environmental toxicity.

One case of hepatoma was diagnosed in a rainbow trout from a Federal hatchery. This is of interest since it was not too many years ago that this tumor was as common as kidney disease and furunculosis.

W. T. Yasutake

BIOCHEMISTRY

The stress of anesthesia with MS-222 and benzocaine

In mammals, certain anesthetics (e.g., diethyl ether) cause a marked stress reaction involving activation of the pituitary-adrenal axis, while other anesthetics, notably the barbiturates, do not.

Preliminary work reported last year indicated that prolonged anesthesia with MS-222 can cause blood chemistry changes in 3-year-old rainbow trout and yearling coho salmon. Since then, further experiments have been done to evaluate the stressor properties of MS-222 anesthesia in yearling rainbows and to evaluate anesthesia with neutralized MS-222 (pH 7), or benzocaine (ethyl p-aminobenzoate), a close chemical relative which shows considerable promise as a fish anesthetic.

Groups of 10 yearling rainbow trout held at 10 C. in water of 20 ppm hardness ($CaCO_3$) were anesthetized with 80 ppm (0.03 mM) of MS-222 or neutralized MS-222 and 50 ppm (0.03 mM) of benzocaine. Neutralized MS-222 (free base form) was as efficient an anesthetic as the sulfonate form. After immobilization, fish were removed at about 1-minute intervals and blood and anterior kidney samples taken.

Analysis of these samples showed that with MS-222 anesthesia metabolic changes occur which are correlated with exposure time. Blood Urea Nitrogen (BUN) levels progressively increase (uremia), a tendency toward hypercholesterolemia occurs, and ACTH production, as measured by interrenal vitamin C depletion, takes place (Figures 12, 13 and 14). The calculated correlation coefficients for the regression of these changes on time are 0.6, 0.6, and 0.7, respectively and are significant.

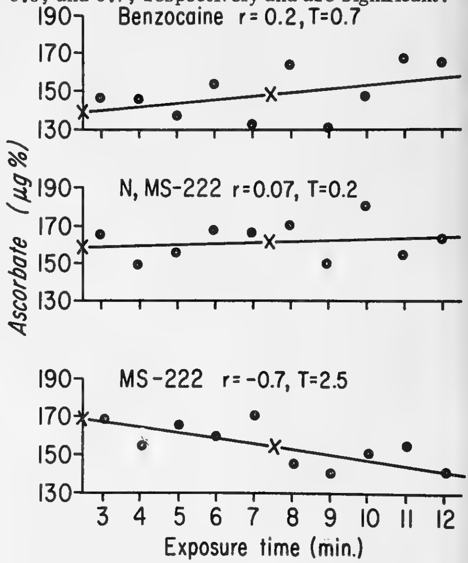


Figure 12.--Interrenal ascorbate levels in anesthetized rainbow trout as a function of exposure time; 10° C., 0.03mM/L. Correlation coefficient (r) and t test for zero slope (T) are given; the MS-222 regression line slope is statistically significant (P = 0.05, T > 2.3) indicating vitamin C depletion with this anesthetic.

These trends in blood chemistry reflect the stress of anesthesia due to the low (3.5) pK of MS-222. Anesthesia with neutralized MS-222 (pH 7, free base) or benzocaine eliminated these disturbances in metabolism, implying that the sulfonic acid moiety of MS-222 is involved. The use of neutralized MS-222 or benzocaine also prevented the initial agitated swimming which occurs when fish are anesthetized with MS-222. In addition, benzocaine anesthesia was subjectively associated with less blood clotting and much less reflex twitching than with the other anesthetics.

There were no significant changes in plasma cortisol, glucose, or chloride with any of the three anesthetics. However, an F test of

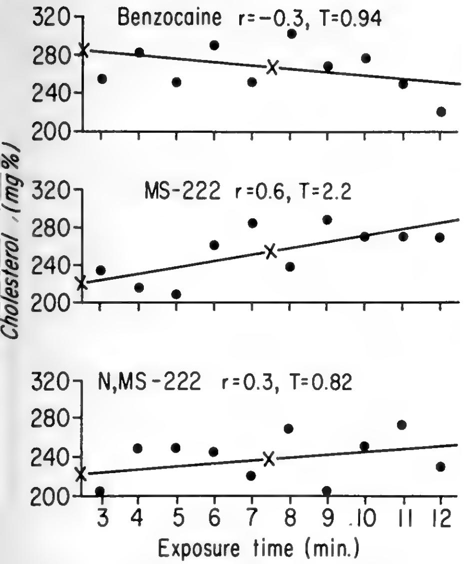


Figure 13.--Plasma cholesterol levels in anesthetized rainbow trout as a function of exposure time; 10° C., 0.03 mM/L. Correlation coefficients (r) and t test for zero slope (T) are given. A moderate hypercholesterolemia, significant at the 93% confidence level, occurred with MS-222 anesthesia.

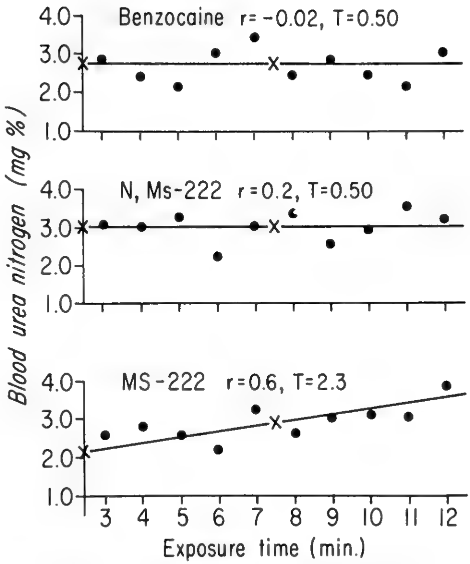


Figure 14.--BUN levels in anesthetized rainbow trout as a function of time anesthetized; 10° C., 0.03 mM/L. Correlation coefficient (r) and t test for zero slope (T) are given. A statistically significant uremia (P = 0.05, T = 2.3) developed when the fish were anesthetized with MS-222.

variance ratios revealed that variability in plasma glucose, cortisone, and cholesterol was greatest when MS-222 was used, while the variability in the chloride and BUN levels was independent of the anesthetic used. For interrenal ascorbate, neutralized MS-222 anesthesia gave the least variable results. Thus, benzocaine or neutralized MS-222 would be the anesthetics of choice for clinical chemistry measurements unless the exposure period was limited to 3-4 minutes or water of sufficient hardness ($CaCO_3$) to buffer the MS-222 to pH 7 could be used.

Crowding stress

The effect of high loading factors on disease susceptibility is well known. However, the responsible metabolic changes have not been studied. To determine some of these, yearling rainbow trout were held at population densities of 1, 3, or 7 lbs/ft^3 for periods up to 35 days. Water temperature was 50° F., dissolved oxygen remained above 8 ppm, and free ammonia (NH_3) was kept below 0.1 ppm; minimum inflow was 0.3 gal/min. Metabolic parameters measured were: plasma cholesterol, glucose, chloride, urea (BUN), cortisol, and total protein. Pronephric vitamin C was also determined.

The data indicated that limited metabolic changes do occur during long-term crowding. Of major interest was the fact that cholesterol and urea levels (which reflect liver function) fell within 3 days to about half their initial values and then slowly returned to "normal" over the 35-day period. Vitamin C levels followed the same pattern but at a slower rate, presumably reflecting the initial hormonal response to crowding, followed by adaptation. The drop in BUN may have simply reflected laboratory diuresis but the initial decrease in cholesterol is unexplained. Blood glucose and chloride levels rose somewhat over the first three days and then showed little change. As expected from the glucose and vitamin C levels, plasma cortisol was somewhat elevated during the first few days but gradually decreased over the 35-day period, again reflecting adaptation to crowding. Total protein levels remained fairly constant over the entire crowding period.

By the end of 35 days, there were no longer any significant differences in blood chemistry among any of the crowding levels implying that the fish were becoming metabolically adapted to the high population densities.

Blood chemistry of the rainbow trout

The normal ranges for numerous blood chemistry parameters have been well established for many of the higher vertebrates and are of proven value as diagnostic tools in human and animal medicine. However, information about the blood chemistry of fishes is fragmentary and is especially meager in the case of the normal ranges to be expected. Following clinical usage, the term "normal range" is the mean $\pm$ two standard deviations and not merely the range of the data.

Yearling rainbow trout (New Castle strain) averaging 100 g in weight were held at 10-13 C. in water of 20 ppm hardness ($CaCO_3$), maintained on the Abernathy diet, and fasted overnight before bleeding. MS-222 or benzocaine anesthesia was used under conditions shown to have no effect on blood chemistry. Results were calculated from approximately 1,400 clinical tests on more than 200 individual samples of blood plasma. In addition, ascorbic acid levels were determined on anterior kidney tissues from 239 fish. By graphically fitting a normal distribution curve to these samples, the mean and estimated normal ranges for a number of components were obtained, based on the assumption that these parameters are normally distributed in fish populations.

The estimated normal clinical chemistry ranges for interrenal ascorbic acid, plasma chloride, cholesterol, glucose, total protein, cortisol, and BUN in the yearling rainbow trout as derived by this statistical technique are shown in Table 1. Corresponding means for immature brown and brook trout are included for comparative purposes.

Table 1.--Estimated normal clinical chemistry ranges for blood and interrenal tissue in the yearling rainbow trout held under thespecified conditions. The normal range is the mean $\pm$ two standard deviations. Mean values for immature brook and brown trout taken from Phillips.

Chemical component	Rainbow trout	Brook trout	Brown trout
Ascorbate µg % (kidney)	102 - 214	-	-
BUN mg % (plasma)	0.9 - 4.5	2.6	2.6
Chloride mEq/l	84 - 132	-	-
Cholesterol mg %	161 - 365	316	402
Cortisol µg %	1.5 - 18.5	-	-
Glucose mg %	41 - 151	70	71
Total protein g %	2 - 6	2.0	2.0

At the present time, these estimates of the normal clinical chemistry ranges for the rainbow trout should be thought of only as a guide to what can reasonably be expected in a population of this age group held under the specified conditions. For example, total protein, BUN, or ascorbate levels outside the ranges given may reflect dietary imbalances rather than pathological processes while cortisol levels can reflect handling stress. However, parameters such as blood chloride (which is involved with blood pH) would be less subject to environmental changes.

Establishing normal clinical ranges for a wider variety of conditions and for additional constituents will provide new tools for determining the optimum environmental requirements of fishes as well as make available disease diagnostic techniques now used only in human and animal medicine. However, it must be emphasized that the normal range is the item of interest, not the mean values by themselves.

Formalin uptake and toxicity in the rainbow trout

Formalin treatments for external parasites have long been used in fish culture operations. Formalin is toxic in some situations but there is no information available concerning the blood and tissue levels of formaldehyde which build up during treatments, the metabolic changes these cause, or on clearance rates following treatments.

Data showing blood formaldehyde levels vs. treatment levels in water of 20 ppm ($CaCO_3$) hardness are shown in Table 2. There is no explanation for the decreased formaldehyde blood levels at higher temperatures; however, these data imply that rainbow trout of this size cannot survive blood levels above approximately 40 ppm in this water supply.

Table 2.--Blood formaldehyde levels as a function of formalin treatment levels and water temperature in yearling rainbow trout. 1-hour exposure.

Treatment	Blood levels (ppm)		
level (ppm)	48° F	56° F	62° F
200	18	15	14
400	22	18	17
800	28	25	21
1600	44	38	29

To learn of metabolic changes induced by formalin treatment, blood glucose, chloride, bilirubin, urea, total protein, and cortisol were measured and correlated with formaldehyde blood levels occurring after 200, 400, 800, 1600 ppm 1-hour formalin treatments.

Blood chemistry changes vs. exposure time are shown in Table 3. The elevated blood glucose levels may be due to formaldehyde interference in the o-toluidine method used. The moderate increase in bilirubin indicates either accelerated hemoglobin breakdown or impaired liver clearance. The downward trend in blood chloride implies but does not prove that metabolic acidosis occurs. However, these changes are on the borderline of statistical significance and additional work will be required before definitive statements can be made.

Table 3.--Blood chemistry changes as a function of time. Yearling rainbow trout, exposed to 200 ppm formalin, at 50° F.

	Exposure time (hours)			
Constituent	0	1	3	6
				6
Chloride mEq/l	124	113	107	87
Bilirubin mg%	0.5	0.8	0.8	1.8
Glucose mg%	173	452	336	285

Preliminary work on clearance times following a standard 200 ppm, 1-hour formalin treatment indicates that up to 3 weeks may be required, at 50 F., to completely eliminate formaldehyde from the blood.

Gary Wedemeyer

THERAPEUTICS

Furanace^{R}(P-7138)

Following the successful experimental control of Chondrococcus columnaris last year, we set out to test the drug extensively under field conditions and expand our laboratory tests. The Dainippon Pharmaceutical Co. Ltd., Osaka, Japan, awarded the Abbott Laboratories the option for distribution of the drug in the Western Hemisphere. The Western Fish Disease Laboratory was selected to coordinate the field testing and approve testing procedures for the Japanese and American Pharmaceutical Companies. Data were compiled for registration with the Food and Drug Administration. The following agencies cooperated in the 1969 tests: Washington Department of Fisheries, Game Commission of Oregon, Fish Commission of Oregon, California Department of Fish and Game, Oregon State University, University of Washington, and several Federal hatcheries.

The myxobacterial diseases were the most thoroughly investigated. Bacterial cold-water disease, columnaris disease, and myxobacterial gill disease were experimentally controlled with a single 1-hour exposure to 0.5 or 1.0 ppm active ingredient of Furanace or to two daily 1-hour exposures. The recommended treatment apparently will be two daily 1-hour exposures at 1.0 ppm.

Therapeutic blood levels were obtained in coho salmon following a single feeding of 25 mg Furanace/kg body weight up to 100 mg/kg. However, with a hard pellet a palatability problem developed with repeated feedings. Consequently, in vivo studies to show control of experimental Aeromonas salmonicida infections were unsuccessful because the fish refused feed following the first administration. Some control was afforded by administering the drug in the water for 2 to 3 consecutive days for 1 hour at 8 ppm, but some toxicity problems were encountered which would contraindicate this method. Robert Garrison of the Game Commission of Oregon reported successful control of Vibrio anquillarum in chinook salmon by feeding the drug at 50 and 75 mg/kg and did not observe any palatability problems with a soft pellet. Dosage at 200 mg/kg appeared toxic and dosages below 50 mg/kg were ineffective.

Furanace has given poor results in controlling protozoan infections. Treatment at 1.0 ppm was ineffective against Costia infestations. Ellis Wyatt of the Fish Commission of Oregon reported that spring chinook salmon infected with Myxobolus insidiosis were administered six 1-hour treatments at 5 ppm and 10 ppm: the drug did not adversely affect the parasite. He also reported that the 10 ppm treatment was toxic to the fish following the fifth exposure.

To determine some of the factors influencing the toxicity of Furanace, coho salmon were exposed to various concentrations of drug, various frequency of treatments, and different water temperatures. Acute toxicity was not observed following a single exposure up to 10 ppm at 70° F., but the mortality rate increased with repeated exposures and with higher dosage levels (Figure 15). In addition, the toxicity increased with an increase in water temperature (Figure 16). In general, the frequency of treatment had influence on toxicity than did the other factors. Leif Marking of the Fish Control Laboratories, using 96-hour standard LC_{50} bioassays, made similar tests and reported similar results. In addition, he found that the drug is more toxic in soft water than in hard water. It was concluded that the drug is not very toxic, especially if exposure time is short.

Further studies were performed to determine the elimination rate of Furanace at 45° and 70° F. Coho salmon were given 3 daily 1-hour exposures to Furanace at 2.0 ppm and tissue residue levels determined for 96 hours. The drug could not be detected in the muscle, blood, kidney, liver, or skin 48 hours after the last exposure at 70° F. The drug was also not detectable in the muscle, blood, or skin after 48 hours at 45° F., but traces were found in the kidney and liver up to 96 hours. Experiments are underway to determine how long the drug is retained in the liver and kidney at 45° F. A preliminary experiment was performed to determine its fate after absorption. Within minutes following treatment, high concentrations of drug were found in the urine and gill washings, suggesting that Furanace crosses cell membranes rapidly and may not be extensively metabolized.

Erythromycin (Gallimycin-50)

A field test was performed at a State of Washington salmon hatchery by Mark DeCew, pathologist for the Washington Department of Fisheries, in cooperation with Abbott Laboratories and the Western Fish Disease Laboratory to determine the efficacy of erythromycin therap for control of kidney disease in chinook salmon. About 40,000 fish received 100 mg/kg/day for 21 days and an equal number of untreated fish served as control. Both groups had about equal disease incidence before treatment. One month following treatment the cumulative percent loss in the untreated group was 2.4 percent and in the treated group, 0.5 percent.

Donald F. Amend

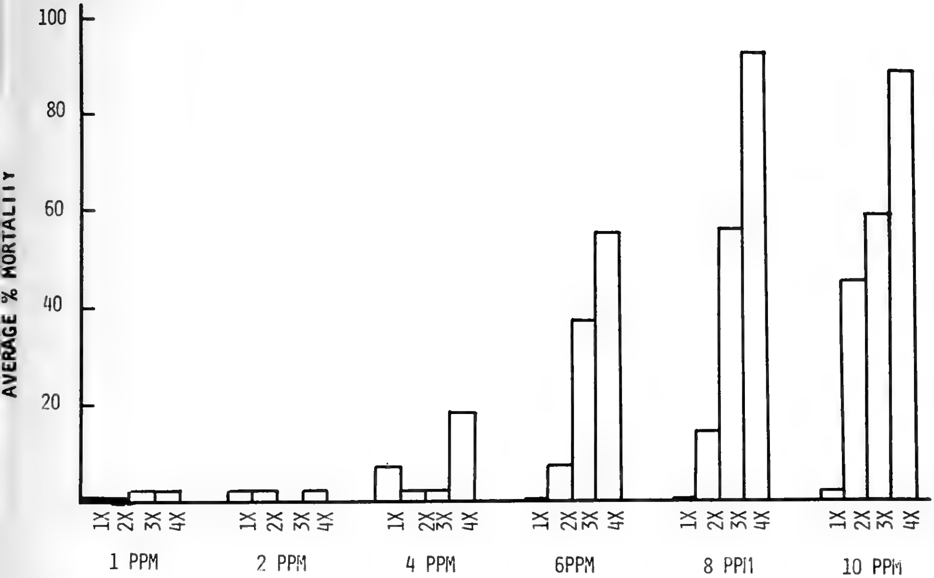


Figure 15.--Average percent mortality from duplicate lots of coho salmon after a 1-hour exposure to various dosage levels of Furanace at 70° F. Fish were given either 1, 2, 3, or 4 exposures 24 hours apart at each dosage level.

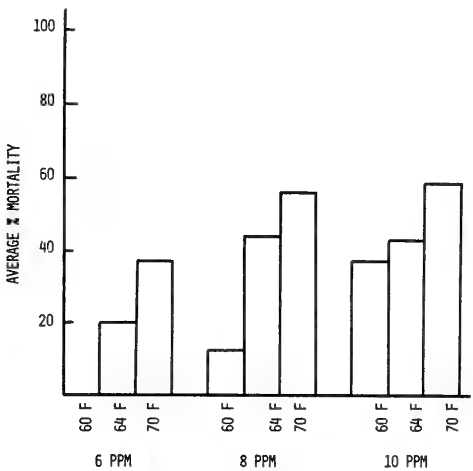


Figure 16.--Average percent mortality of coho salmon showing the effect of temperature on Furance toxicity following 3 daily 1-hour exposures at 3 dosage levels.

EASTERN FISH NUTRITION LABORATORY

Cortland, New York
Arthur M. Phillips, Jr., Director

The Cortland Laboratory is operated cooperatively with New York State and Cornell University. Research results, published annually by the State in a numbered series, "Fisheries Research Bulletin," may be obtained from the laboratory or the New York Conservation Department.

HIGHLIGHTS

Trout fed a fat-free diet had abnormal-appearing livers that varied from creamy white to light brown. Those fed a similar diet fortified with ethyl linolenate had "normal" red-colored livers.

The erythrocytes of brook, brown, and rainbow trout contained between 3 and 6 times more inorganic phosphorus, from 2 to 3 times more cholesterol, and between 2.9 and 3.6 milligrams percent less lipid phosphorus than in human red cells.

A combination of supplemental choline and niacin helped control body fat deposition in trout fed a fat-enriched diet.

Massive doses of vitamin E reduced the hematocrit and the growth of brook trout.

Thiamin is utilized by fish much the same as by higher vertebrates. A thiamin deficiency in trout resulted in a predictable upset of carbohydrate metabolism.

The onset of functional sexual maturity of male brook trout was influenced by exposure to continuous illumination or to continuous darkness. Continuous light accelerated functional maturity by about 14 weeks and continuous darkness retarded onset by 6 weeks.

Δ^1-Testololactone (Teslac) increased trout growth when their eggs were water-hardened in a solution of the compound.

Oxytocin injections into sexually mature brown trout did not induce spawning or spawning activity.

Acid-soluble non-ionic phosphorus compounds were formed from dietary phosphate by both brown and rainbow trout and were used in the distribution of phosphorus to their tissues.

Heavy metals significantly disabled the cation uptake mechanism of brown trout; alkaline earth cations excreted a protective action against toxic effects of copper, zinc, cobalt, and nickel.

DIET COMPOSITION AND THE GROWTH, SURVIVAL, AND QUALITY OF HATCHERY TROUT

The influence of essential fatty acids on brook trout growth and lipid metabolism

Salmon and trout require certain polyunsaturated fatty acids for proper dermal pigmentation, body growth, prevention of dermal lesions, and survival. Our recent work showed a syndrome indicative of an essential fatty acid deficiency in fish fed hydrogenated safflower oil, suggesting that changes caused by hydrogenation had resulted in this condition.

Duplicate groups of fingerlings, initially weighing an average of 4.36 grams, were held for 12 weeks in troughs supplied with water at an average temperature of 12.4° C. and for an additional 12 weeks at a constant water temperature of 8.3° C. The warm water supply became contaminated with freshwater crustacea and, although the incoming water was filtered by fine-mesh screens and cloth, some of the smaller crustacea were eaten by the trout. To overcome this difficulty a crustacea-free spring-water supply at a constant temperature of 8.3° C. was substituted at the end of 12 weeks.

The fish were fed either a fat-free semi-purified diet in which the major source of energy was sucrose, or a similar diet plus either 1 percent of ethyl linoleate or 0.1 percent methyl linolenate.

There was an apparent difference in terminal body weights, but the differences were not statistically significant. Mortality increased slightly in all groups during the final 12 weeks of the study. There were no differences in mortality among the fish fed different diets.

Terminal samples of liver were frozen in dry ice, weighed, and held at -15° C. for liver fatty acid analysis. Other terminal samples were preserved for histochemical analyses.

Expressed as a percent of body weight, the liver weights for fish fed the fat-free diet, the fat-free diet supplemented with ethyl linoleate, methyl linolenate, or ethyl linoleate plus methyl linolenate were 2.09, 2.33, 2.75, and 2.80, respectively. These are significant differences.

The livers differed in gross appearance. Trout fed the fat-free diet without supplements had terminal livers that varied from creamy white to a very light brown or "straw" color; those fed ethyl linoleate had "normal" red-colored livers; and those fed methyl linolenate had light brown livers, some similar to those from fish fed the control diet. Some livers from fish fed both linoleate were almost normal red but others were mottled with small white areas.

Results of the histological and histochemical tests, conducted by Dr. Roger L. Herman, physiologist, Eastern Fish Disease Laboratory, showed that all livers except controls, were highly vacuolated. There was a very strong positive reaction for polysaccharide storage (probably glycogen) with the periodic acid-Schiff (PAS) stain, and Oil Red O and Sudan Black stains showed variable lipid storage. There was no indication of ceroid type pigments.

Fixation and storage in formalin and water precluded a true picture of liver glycogen storage. The sucrose level of the diets was 37 percent and the livers probably would, therefore, contain high glycogen levels.

Hugh A. Poston and Donald L. Livingston

CHEMICAL COMPOSITION OF TROUT BLOOD

Because it is nucleated and can be readily isolated, the trout erythrocyte could serve for studies of cell metabolism. Although there is a good deal of information on the chemical composition of human erythrocytes and most domesticated mammals and fowl, there are little data on the chemistry of the trout erythrocyte.

This report is the second in a series of investigations to establish "normal" values for a number of chemical constituents in trout erythrocytes. The initial report dealt with cation distribution of mature brown and brook trout. This report concerns the phosphorus, total cholesterol, and total free fatty acid distribution in mature brown, brook and rainbow trout.

Two-year-old trout were used. Five pooled blood samples (composed of 1.0 milliliter of blood from each of two trout) were taken from each species. The erythrocytes were isolated and analyzed for the constituents listed in Table 1.

Table 1.--Distribution of phosphorus, total cholesterol, and free fatty acid in the erythrocytes of three species of trout

Component	Milligrams per 100 milliliters of erythrocytes1/		
	Brown trout	Brook trout	Rainbow trout
Inorganic phosphorus	12.70 ± 1.360	14.70 ± 1.330	14.10 ± 1.200
Nucleic acid phosphorus	9.40 ± 0.045	0.29 ± 0.036	0.37 ± 0.039
Phospho-protein phosphorus	3.00 ± 0.375	2.60 ± 0.412	1.80 ± 0.482
Lipid phosphorus	9.00 ± 1.110	8.80 ± 1.610	8.30 ± 1.290
Barium fraction I	51.50 ± 5.820	53.40 ± 2.680	55.80 ± 3.570
Barium fraction II	1.50 ± 0.180	2.10 ± 0.150	2.60 ± 0.240
Total cholesterol	334.00 ± 15.150	279.00 ± 37.820	241.00 ± 19.490
Free fatty acid	106.30 ± 5.920	82.20 ± 7.640	98.90 ± 11.700

1/ All values ± 1 standard deviation.

Inorganic phosphorus levels were 3-6 times higher than those reported for humans and total cholesterol levels were 2-3 times those of human red cells. The total free fatty acid levels were about one-half that of human red cells. Lipid phosphorus levels were from 2.9 to 3.6 milligrams percent lower than in humans. The phosphoglycerate fraction was somewhat higher and the hexose mono-phosphate fraction somewhat lower than those reported for human red cells. No comparative data were available for nucleic acid and phospho-protein phosphorus.

The apparent differences among the three species may or may not be significant. The experiment does not lend itself to the establishment of the significance of these differences.

Thomas H. McCartney

Characterization of serum protein electrophoretic patterns of trout reared in continuous darkness or continuous illumination

This study was designed to determine differences in electrophoretic patterns of serum proteins in brook trout reared in continuous light, continuous darkness, or continuous simulating natural seasonal photoperiods. Blood serum was obtained periodically. Notes were made of the sex of each fish sampled, and the stage of maturity of its gonads. The serum was stored at -15° C. until electrophoresed by the Disc Polyacrylamide Gel method. The serum samples were being analyzed by the use of a microdensitometer, when the machine failed. Analyses will be completed later.

Gross qualitative examination of the electrophoretic columns showed that, as the female brook trout of each controlled lighting environment approached functional sexual maturity, a new protein fraction appeared in their serum; this was not present in the male trout serum. This protein fraction has very slow mobility and probably is a lipoprotein.

Seasonal serum protein electrophoretic properties of brook, brown, and rainbow trout

A study to compare the seasonal serum protein electrophoretic properties of brook, brown, and rainbow trout was initiated in late May, 1968. One hundred each of the three species, approximately 18 months old, were placed in a production raceway and fed a meat-dry meal diet daily throughout the study.

Blood samples, obtained by severing the caudal peduncle, were taken at approximately monthly intervals until sampling was terminated in early November, 1969. Six fish per species were sampled monthly during most of the study, but available numbers of fish reduced the number sampled to either four or five fish during the last six months. The species, sex, weight, fork length, and stage of functional maturity and gonad condition were recorded for each fish sampled.

Serum samples were held at -15° C. until electrophoresed. Gross inspection of the completed electrophoretic columns shows the appearance of a protein fraction with low mobility in the female serum of each species as they approached functional sexual maturity.

Hugh A. Poston

VITAMIN REQUIREMENT OF TROUT

The interrelation of choline, niacin, methionine, and tryptophan on lipid metabolism of brown trout

In our previous experiments, we have attempted to prevent increased body fat in trout fed isocaloric, fat-supplemented diets by using different sources of food fat, manipulating protein sources, altering protein quality, and supplementing with vitamins and amino acids. In last year's work, supplementing fat-enriched diets with either choline or methionine did not prevent or reduce increased body fat. It may be that supplementation of more than one dietary ingredient before the fat-enriched meat-dry meal diet will produce less body fat.

With this possibility in mind, we fed brown trout a fat-enriched diet supplemented with choline, niacin, methionine, and/or tryptophan.

Fingerlings were held at two water temperatures (constant 8.3° and average 12.4° C.) for 20 weeks. Duplicate troughs of fish were fed, at each water temperature, isocaloric meat-dry meal diets that varied in proportions of meat (50 or 36.5 percent) and dry meal (50.0 or 35 percent) and in percent of calories supplied as protein (67 or 46 percent) and as fat (25 or 46 percent). These diets either were supplemented with 6 percent safflower oil or not at all. The fat-enriched diets were fortified with varying combinations of niacin (0.05 percent of diet) and/or choline, methionine, and tryptophan (each at 0.5 percent of diet), or not at all.

Terminal livers were frozen in dry ice, weighed and held at -15° C. until analyzed for fatty acid composition (by gas liquid chromatography) and total lipids.

Body size. There were no statistically significant differences in average terminal body weights among the groups held at either water temperature. However, fish fed the low protein diet (46 percent of calories as protein; 18.8 percent protein) containing methionine as the sole supplement in the colder water showed an apparent depression in body weight that approached statistical significance.

Terminal average body weights of all fish in the warmer water were significantly greater than those in the colder water.

Percent of body fat. Fish fed diets with supplemental fat had increased body fat at both water temperatures. Those fed the diet that furnished an increased proportion of calories as protein contained less fat than fish fed the other diets at the two water temperatures. However, those fed the high-protein diet had significantly higher levels of fat when held in the warmer water than those fed the same diet in the colder water.

Trout fed the fat-enriched diet supplemented with methionine only, in the warmer water, had significantly more fat than those fed the diets supplemented with methionine, choline, and niacin or with choline and tryptophan at the same water temperature.

Fish fed the fat-enriched diet supplemented with choline and niacin in the colder water contained less fat than those fed any other fat-enriched diet at that temperature. Their level of body fat was as low as that in the fish fed the high-protein diet in the warmer water.

Total liver lipids. Fish held in the warmer water and fed the high-protein low-fat diet had the least lipids per gram of liver. The pattern of lipid deposition in fish held in the warmer water does not show any conclusive effects of the amino acid and vitamin supplementation.

In fish held in the colder water the addition of the individual amino acids and vitamins, and choline and niacin, to the fat-enriched diet reduced lipid deposition.

Liver fatty acid composition. Increasing the proportion of calories furnished by dietary fat from 25 to 46 percent, and the consequent reduction of calories furnished by protein from 67 to 46 percent, caused a 182 percent increase in liver linoleic acid in the warmer water and a 162 percent increase of the same fatty acid in the colder water. This dietary change also reduced oleic acid by approximately 50 percent at both water temperatures. Linolenic acid, never present in more than negligible amounts in livers of fish in the warmer water, doubled in percentage of the total fatty acids after the fish were fed the fat-enriched diet in the colder water.

The addition of niacin, methionine, or tryptophan individually to the fat-enriched diets reduced palmitic acid at both water temperatures, and the supplemental mixture of choline, niacin, methionine, and tryptophan caused a similar decrease in the colder water.

The addition of choline and niacin, or choline or niacin alone, to the fat-enriched diet increased the level of linoleic acid at both water temperatures, and methionine increased the level in the warmer water.

Water temperature. With a few exceptions, the liver fatty acid composition was similar at the two water temperatures. Larger amounts of linoleic acid and some of the longer-chained, more highly unsaturated acids generally were present in fish fed a given diet in the colder water. Clupanodonic acid, not present in measurable amounts in livers from any fish held in the warmer water, was found when diets were supplemented with amino acids and vitamins in the colder water.

Summary. At both water temperatures fat supplementation with safflower oil increased the body fat without significant body weight increase. A mixture of supplemental choline and niacin reduced body fat deposition when the fat-enriched diet was fed in the colder water, but neither choline nor niacin individually significantly affected body fat levels. Choline and niacin reduced body fat in the warmer water when the mixture was added to the fat-enriched diet that also contained supplemental methionine. These results indicate a combination of supplemental choline and niacin is needed to help control body fat deposition in trout fed our fat-enriched diet.

The increased liver linoleic acid, and the concomitant decrease in other liver fatty acids for fat-enriched diets at both water temperatures is a reflection of the high linoleic acid content of safflower oil.

The reason for the boost in liver linoleic acid by the individual supplementation of choline, niacin, and methionine, and of choline and niacin is not clear, but possibly involves the biological donation of methyl groups.

Hugh A. Poston and Donald L. Livingston

Massive doses of vitamin E in brook trout diets

Previous work at this laboratory has shown that massive doses of the fat-soluble vitamins A and D produce physiological changes in brook trout.

The study described here was designed to determine effects of high dietary levels of the fat-soluble tocopherol (vitamin E) on brook trout hematocrit, liver lipid content, liver fatty acid composition, and body growth.

Duplicate groups of fingerlings, initially weighing 1.07 grams, were fed either a control diet (semi-purified Wolf diet) that contained 50 milligrams of dl-alpha tocopherol per 100 grams of dry diet, or the control diet plus an additional 450 milligrams of dl-alpha tocopherol per 100 grams of dry diet. The experiments ran 20 weeks at an average water temperature of 12.4° C.

Hematocrits were taken at the end of 14 weeks and upon termination. Terminal liver samples were frozen in dry ice, weighed and held at -15° C. until analyzed for total lipids and fatty acid composition.

Compared with hematocrits from the controls, hematocrit values for trout fed excess tocopherol showed a 13 percent reduction at the end of 14 weeks, and 18 percent at the end of the experiment.

Average lipid content per gram of liver was over 26 percent higher in fish fed excess tocopherol than in those fed the control diet.

Livers from fish fed the control diet were heavier than those with excess tocopherol. The hepatosomatic index (liver weight expressed as a percent of body weight) was 1.73 for the control diet and 1.54 for the excess tocopherol.

Liver linoleic acid, expressed as a percentage of the total liver fatty acids, was 25 percent lower with excess tocopherol. No other differences in fatty acids were detected.

The average body weight of fish fed excess tocopherol was 6.5 percent less than that of the control. Neither diet caused unusual mortality.

Lowered hematocrit, changes in liver chemistry, and growth depression suggest that high levels of dietary alpha-tocopherol exert an adverse effect. Earlier work at this laboratory showed that brook trout fingerlings fed 125 milligrams of dl-alpha tocopherol per 100 grams of dry semi-purified diet had higher hematocrits than those fed 20 milligrams of tocopherol per 100 grams of drydiet. The optimal level, in the semi-purified diet used in this study, appears to lie between 50 and 500 milligrams of tocopherol per 100 grams of dry diet.

The specific metabolic effects of high levels of dietary tocopherol on erythropoiesis and erythrocyte integrity have not yet been measured.

Hugh A. Poston and Donald L. Livingston

<u>A thiamine deficient diet and its effects on serum and liver chemistry of fingerling brook trout</u>

Thiamine pyrophosphate is a required cofactor in two major biochemical transformations during carbohydrate metabolism: (1) for the formation of acetyl coenzyme A from pyruvate and coenzyme A, and (2) for the transketolase reaction in the operation of the pentose phosphate pathway for glucose oxidation. In higher animals the pentose phosphate cycle, among other tissues, is active in erythrocytes and liver cells and in rats the transketolase activity of red cells has been used as an indicator of thimine deficiency. Other studies have shown that the pentose phosphate pathway for glucose metabolism may be of more importance in trout liver than in that of other animals.

Based upon trout growth and mortality rates thiamine is well established as an essential nutrient but the biochemical changes caused by thiamine deficiency are not well defined.

In this experiment, we fed fingerling brook trout a thiamine-free synthetic diet for 20 weeks. At the end of that time liver samples were analyzed for sedoheptulose-7-phosphate, DNA, pyruvic acid, glucose, fructose, and organic phosphorus, and blood serum samples for glucose, fructose, and inorganic phosphorus.

The liver data show the effect of the thiamine deficiency on the pentose phosphate pathway of glucose metabolism. The amount of sedokeptulose-7-phosphate (a product of the transketolation of xylulose-5-phosphate and ribose-5-phosphate) in the livers was only two-thirds that of the controls, indicating that absence of thiamine reduced transketolase activity. The liver DNA level was only one-half that of the controls. A significant amount of the ribose for nucleic acid synthesis arises from a reversal of the pentose phosphate pathway or a reversal of the transketolase reaction. Liver glucose and pyruvate were reduced in the thiamine-deficient trout, indicating a general suppression of glucose metabolism in the absence of thiamine. Liver fructose, however, was not affected, and no satisfactory explanation is apparent. The inorganic phosphorus of the liver was not altered by thiamine deficiency.

It was not possible to obtain sufficient serum to measure the amount of pyruvate in the bloodstream. An increased level of pyruvate is an indication of thiamine deficiency in other animals. However, the increase of serum glucose and fructose levels show that there was a marked alteration in their carbohydrate metabolism. The serum inorganic phosphorus decreased.

The data indicate that thiamine is utilized in fish in much the same fashion as in higher vertebrates and that the pentose phosphate cycle may indeed be of greater significance to trout metabolism than to the metabolism of higher vertebrates.

Thomas H. McCartney

PHYSICAL FACTORS AND GROWTH OF HATCHERY TROUT

Effect of continuous darkness or continuous light on sexual maturity of brook trout

We commenced a series of experiments in March, 1966 to study the effect of continuous light and continuous darkness on brook trout growth and sexual maturity.

Fingerlings, initially averaging 0.465 grams, were stocked in each of three light-controlled chambers. One chamber was kept in total darkness except for infra-red illumination supplied through an 87C Wratten filter, giving 0.32 percent transmission of 8,000 A° wavelength light. Infra-red illumination was used for observation with the aid of a mechanical viewer, and for biweekly or monthly weighings.

A second chamber received light continuously from two 60-watt and one 100-watt incandescent lamps that provided 16 foot-candles at the water's surface. A spectral analysis, with a radiospectrograph and head, showed that the peak wave lengths of light at nine inches beneath the water's surface were in the very deep red. A battery-powered lamp provided light in the case of electric power failure.

The third chamber had light of the same intensity as the second chamber, but the lamps were switched on and off daily by means of a photoelectric control as daylight and darkness approached. The fish in the third chamber were thus exposed to a daily period of artificial light that varied and was comparable to the hours of natural daylight.

Each chamber contained one 210-gallon tank supplied with constant-temperature water at 8.3° C.

The fish, initially numbering 2,150 per tank, were reduced in number periodically to prevent overcrowding. Fish removed at the end of 72 weeks were fin clipped for identification, and held for spawning in a production raceway.

The results of the first 94 weeks of the study were reported in the Cortland Hatchery Report for the Year 1967. The following is a brief review of the earlier reported work.

When the surplus fish were transferred from the three experimental chambers to a production raceway at the end of 72 weeks, male trout from continuous light had enlarged, milky white testes and copious semen with viable spermatozoa. Males from the chamber that simulated natural photoperiods had testes slightly larger than those from males in continuous darkness, but there was no indication of semen from fish in the latter two groups. Females held in continuous light had eggs several times larger than those in ovaries of females in continuous darkness or simulated natural light.

We expected the fish held in continuous light to spawn much earlier than those held in either of the other two light environments. The trout placed in the production raceway did spawn at different times, as predicted on the basis of gonadal development. Those held in continuous light first spawned at about 6 weeks; those from simulated light first spawned at about 9 weeks; and those from continuous darkness about 11 weeks after transfer to the raceway.

The time interval to first spawning in the trout remaining in continuous light, after surplus fish were transferred to natural photoperiods, was 12 weeks, compared with 6 weeks required for the fish that had been placed in natural light from the continuous light chamber. Females transferred from continuous darkness to natural photoperiods and light spawned 5 weeks before those fish held in continuous darkness through spawning.

The delay in spawning, in fish held in continuous light, compared with that in fish switched from these conditions to natural light and photoperiods, suggests an insufficiency of a release factor(s) necessary for the spawning of ripe eggs.

Continuation through the second reproductive cycle. The apparent delay in spawning time in fish held in continuous light, the apparent lack of effect of artificial light on the spawning date of fish held in light-controlled environments, and the reports in the literature suggesting that functional sexual maturity of trout undergoing gametogenesis for the first time is not affected by variation in photoperiods, prompted us to hold the same fish in their light-controlled environments until they reached spawning condition a second time.

Males and females were examined periodically for external and internal indications of sexual maturity.

On April 11, 1968, several males in continuous light had a well-developed "kipe" of the lower mandible and on April 25, 1968, 24 of the remaining 36 continuous light males had copious semen. The majority of males in this group continued to have semen until mid-July, 1968.

Males in the chamber with simulated natural light first showed copious semen, for their second season, on August 29, 1968, and continued to have semen through January 7, 1969. Eight out of 27 males in continuous darkness had reached functional maturity for their second season by October 10, 1968. However, the majority of the males in continuous darkness did not emit semen until December 5, 1968. Semen could still be taken from most of these males on March 7, 1969, but not on April 3, 1969.

One female held in continuous light could be stripped of normal eggs on September 9, 1968. However, no other female in the continuous light group had ripe eggs until November 7, 1968. The majority of the females held in the simulated natural light environment were spawned from October 10 through November 7, 1968. The first eggs were taken from a female in constant darkness on December 5, 1968, with the majority of eggs from this group being taken during January, 1969.

The ovaries of females held in continuous light, and of those held in simulated natural light, contained a new third crop of developing eggs that ranged from less than one millimeter to over two millimeters in diameter on April 3, 1969. No sizeable new eggs could be detected in the fish held in constant darkness.

All surviving fish were removed from the station on April 14, 1969.

This study shows that the time of onset of functional sexual maturity (that is, the time at which copious semen can be expressed) of male brook trout in their second reproductive cycle can be influenced by exposure of the fish to continuous, uninterrupted illumination or by continuous darkness. Exposure of males to continuous light accelerated onset of functional maturity by almost 14 weeks. Exposure of males to continuous darkness retarded onset of functional maturity by 6 weeks.

Exposure of males to uninterrupted illumination apparently shortened the duration of functional maturity in their second reproductive cycle. Copious semen could be expressed from males exposed to continuous light for almost 12 weeks, as compared with almost 19 weeks for males exposed to photoperiods simulating the natural environment, and 21 weeks for males in continuous darkness.

Males in each of the controlled lighting environments became functionally mature before the females. However, the males under continuous light were void of copious semen for 16 weeks before the first eggs were taken from females in continuous light. Onset of functional maturity in the males and females exposed to constant darkness or to simulated natural light was better synchronized.

Duration of functional sexual maturity for the males in their second reproductive cycle was more prolonged in each of the three experimental groups than the 6 weeks reported for male brook trout in the literature.

Exposure of female trout to continuous light or to continuous darkness apparently retarded onset of functional maturity in their second reproductive cycle by approximately one month and two months, respectively, compared with conditions simulating natural light.

The fact that eggs were not stripped from many of the females during their first reproductive cycle possibly altered their second reproductive cycle. Nevertheless, a majority of females under simulated natural light spawned when brook trout are usually spawned under natural light at Cortland.

Hugh A. Poston and Donald L. Livingston

TROUT ENDOCRINOLOGY

Exposure of brook trout eggs to an androgenic-like preparation

Δ^1-Testololactone (Teslac) is a compound that is non-hormonal in routine bioassays for endocrine activity. It does, however, increase the rate of weight gain in mice and rats, following subcutaneous injection on the first day of the animal's life. It also promotes growth in amphibians following the incubation of eggs in its presence.

Our interest was the possibility that trout from eggs water-hardened in a low concentration of Δ^1-Testololactone, would grow more rapidly than those from non-treated eggs.

With this possibility in mind, we water-hardened duplicate groups of 5,000 fertilized brook trout eggs for 30 minutes in beakers of distilled water containing 0.85 percent sodium chloride and 10 p.p.m. of Δ^1-Testololactone that had been dissolved in a 1:1 solution of ethyl alcohol and distilled water. Duplicate control groups of 5,000 fertilized eggs were water-hardened in distilled water containing 0.85 percent sodium chloride and an amount of ethyl alcohol comparable to the level to which the treated eggs were subjected. All eggs were incubated in flowing 8.3° C. water.

Equal numbers of progeny from each lot of eggs were retained in similar troughs supplied with water at a temperature of 8.3° C. The fish were weighed initially and at intervals thereafter to observe their growth. The numbers of fish were reduced periodically to prevent overcrowding.

There was no difference in fertility, time of "eye-up," or percent hatch between the treated eggs and their controls.

At the latest weighing, trout from the treated eggs weighed an average of 13.2 percent more than trout from the control group, a significant difference. There was close agreement between replicate troughs within the treatment groups.

The surviving fish are being held and observed for growth and possible effects of the treatment upon reproduction.

Hugh A. Poston

Injections of oxytocin on spawning female brown trout

It has been reported that injections of oxytocin, a pituitary hormone that stimulates contraction of smooth muscles of the uterus and mammary glands of mammals, will initiate the "spawning reflex" in *Fundulus heteroclitus*. With the possibility that exogenous oxytocin will also induce spawning activity in trout, we studied the effect of injections of synthetic oxytocin on the activity and behavior of sexually mature male and female brown trout.

Four 4-year-old male brown trout, from which semen could be expressed, and four 4-year-old female brown trout, from which eggs could be stripped, were transferred from a production raceway to a trough supplied with water at a temperature of 8.3° C.

A synthetic preparation of oxytocin, which contained 20 U. S. P. units of oxytocic activity per cubic centimeter, was injected either intraperitoneally or intramuscularly into the male and female brown trout, in dosages varying from one and one-half to ten cubic centimeters. The recipients were returned to the trough and observed for release of semen or eggs, and for unusual behavior such as the sigmoid-like posture displayed by Fundulus heteroclitus after oxytocin injections.

No injected fish spontaneously expelled its eggs or semen during 6 hours of close observation. None exhibited unusual body shapes or positions.

All recipients, regardless of route of oxytocin administration, remained in a post-injection state of quiescence, during which they offered no resistance to handling; they had returned to a normal state, in which they resisted touch or capture, by the following day. This quiescent condition possibly was induced by the 0.5 percent chlorobutanol and/or the 0.25 percent acetic acid contained in the oxytocic preparation.

These results indicate that injections of oxytocin into sexually mature brown trout do not induce spawning or spawning activity.

Hugh A. Poston

MINERAL METABOLISM

Phosphorus metabolism, comparing rainbow and brown trout with known metabolism of brook trout

Our earlier studies with brook trout have shown a conversion of inorganic phosphorus from capsulated artificial foods into organic phosphorus compounds within the tissues. Generally, less than 10 percent of the food phosphate was found so converted, but analysis of the organic phosphorus components showed a significant amount of acid-soluble non-ionic phosphorus (probably adenosine polyphosphates) that could have been involved as a carrier that distributed the dietary phosphorus to the tissues. In the tissues of the brook trout digestive tract and muscle, a portion of the transported dietary phosphorus was recovered in the lipid, protein, and nucleic acid phosphorus fractions, within one day after feeding.

Other experiments, however, have shown that the great preponderance of phosphate from the food is recovered in the skin and skeletal tissues, where it remains through at least four days after feeding. Generally, these analytical results indicate an extremely efficient utilization of dietary phosphate. To establish the general existence of these efficient phosphorus metabolism mechanisms, yearling brown and rainbow trout were fed either 0.5 or 2.0 milligrams of dietary phosphorus (as phosphate) that had been labeled with phosphorus-32. Labeled phosphorus in the skin and skeleton, and in the acid-soluble ionic, acid-soluble non-ionic, lipid, nucleic acid, and protein phosphorus fractions of homogenates of the digestive tract and muscle tissues were measured at one-half, one, two, and four days after feeding.

Recoveries of labeled phosphorus. As with yearling brook trout, the utilization of dietary phosphate by both brown and rainbow trout was efficient from both dietary levels, with conversions approaching unity and with recovery of labeled phosphorus generally proportional to dietary level. At one-half day after feeding the food mass was essentially located in the stomach with considerable distribution of the absorbed labeled phosphorus to the other tissues of the fish already having occurred. One day after feeding the food mass had passed into the large intestine and the feces showed the presence of labeled phosphorus. The digestive tract contents were clear two days after feeding.

A species difference between rainbow trout and brown and brook trout was evident before the four-day samples could be taken. The rainbows showed distress in the aquaria, probably from forced feeding of capsules, and, as has been observed in numerous other studies, the distress resulted in their deaths before the end of the four days.

Except for this difference, which is only an artifact of the experiment, the two species responded like brook trout in their distribution and retention of the dietary phosphate, with significant quantities of the labeled phosphorus being recovered in the skin and skeleton within one-half day after feeding and with the skin again proving to be a major repository. The phosphorus incorporated into the structural tissues of the trout was essentially retained by the brown trout through four days after feeding and by the rainbow trout through the two or three days they survived.

Distribution of phosphorus. As with brook trout, recoveries of labeled phosphorus in the acid soluble non-ionic fractions occurred as early as one-half day after feeding. Most of this recovery was in the homogenates of digestive tract tissues. Approximately the same amount of labeled phosphorus was recovered in the acid-soluble ionic fraction. Conversion of the ionic phosphate of the food to a non-ionic acid-soluble form appears to be a significant and efficient mechanism for phosphorus utilization by these three species. Much less labeled phosphorus was in the other organic fractions of the digestive tract at this early period after feeding.

At this time in the muscle tissue homogenates, significant quantities of labeled ionic phosphorus were recovered, but practically no labeled phosphorus in any organic form. A mechanism, therefore, for direct absorption of phosphate from the stomach had been functioning, but the presence of large amounts of the acid-soluble non-ionic labeled phosphorus indicates that probably the direct passage of phosphate through the membranes of the stomach to the blood stream is not the only mechanism at work. The quantity of acid-soluble non-ionic labeled phosphorus in the muscle tissues did increase with time after feeding, but this increase was not commensurate with the amount of inorganic, ionic labeled phosphorus found in the muscle tissues. The recovery of acid-soluble non-ionic labeled phosphorus in the digestive tract tissues continued through the two or four days of the experiment, again indicating some function of this form of phosphorus in the storage and transfer of phosphate from the diet to the tissues.

Only small amounts of labeled phosphorus were recovered in the lipid, nucleic acid, and protein phosphorus fractions of both the digestive tract and muscle tissue homogenates. Most of this small quantity was found in the lipid fraction of the digestive tract soon after feeding. Only at two to four days after feeding did significant recovery of ^{32}P-labeled material in the nucleic acid and protein functions occur.

Phosphorus utilization. These recoveries in the organic fraction, plus the retention of the labeled phosphorus in skin and skeleton, indicate a considerable utilization of dietary phosphate, either by exchange to replace structural phosphorus in the body and thus maintain the fish during food deprivation, or by actual accretion to form new tissue structures. The labile inorganic phosphorus from the single capsule fed each fish is rapidly transported to the repository tissues with the concomitant synthesis of acid-soluble non-ionic phosphorus compounds in the digestive tract tissues. This process apparently mediates the transfer of the dietary phosphorus to the tissues of the skin, skeleton, and muscle. From the long retention of labeled phosphorus in digestive tract tissues in both labile and depot forms, it appears that brown and rainbow trout also have efficient coupling and storage mechanisms for maintaining regulated and efficient phosphorus metabolism.

Henry A. Podoliak and Alphonse S. Smigielski

Effects of divalent cations (alkaline earths and heavy metals) on calcium storage in skeleton and skin by trown trout

In aquarium studies, brown trout have proved most resistant to deleterious effects of some abrupt and extreme water chemistry changes. Their resistance to these changes is correlated with the presence of adequate calcium hardness.

In this experiment calcium-45 labeled, reconstituted water provided a means, through a tracer study, of establishing and measuring calcium uptake, exchange, and storage in the skeletal and skin tissues of fingerlings, and thus observing the effect of water chemistry changes on the ionic homeostasis of the brown trout that survive the challenging environmental conditions.

Heavy metal ions were used in the aquarium water to provide a toxicity stress. Water hardness was provided by alkaline earth metals other than calcium to allow measurement of any protective actions (antagonisms) of these divalent cations against this toxicity stress.

The heavy metals were used in two synergistic combinations. The first, of high toxicity, was a combination of 0.1 ppm of copper and 1.0 ppm of zinc. The second, of intermediate toxicity, and greater concentration, was 1.0 ppm of nickel and 10.0 ppm of cobalt.

The balanced reconstituted water contained 1.0 millimole per liter of both sodium and calcium, with either no other cations, or with a supplemental 1.0 millimole per liter of calcium, magnesium, strontium, barium, or potassium (an antagonistic monovalent cation), all as chloride salts. The fish were held for two days in ozonized labeled water at 10° C., after which their gills, skin, skeleton, and remainder tissues were analyzed for radioactive calcium.

No deaths occurred in the aquarium waters without the added heavy metals. Neither alkaline earth nor potassium ions appeared to stress the brown trout. All four of the added alkaline earths significantly depressed the rate of calcium absorption from the water to about two-thirds of what it had been without these added cations. The alkali potassium ion had no reducing effect on calcium absorption, but actually stimulated calcium absorption by about 20 percent. The distribution of calcium to the different tissues was unaffected by any of these added ions. Most of the labeled calcium was recovered in the skin tissues, although the greatest concentration of labeled calcium, per gram of tissue, was in the bones, where proportionately twice the concentration of that in the gills and skin was measured.

Copper and zinc. All five added ions effectively reduced deaths when the high toxicity heavy metals were present in the waters. Without enrichment the trout died between 24 and 36 hours in the toxic water. No deaths occurred in waters enriched with calcium, strontium, or barium.

The presence of copper and zinc reduced the amount of labeled calcium recovered in the tissues to about one-fifth of that absorbed in the non-toxic water. Potassium again stimulated the absorption of labeled calcium, but now the supplemental alkaline earths only slightly reduced the calcium exchange when this exchange was so highly impaired by the presence of the toxic heavy metals.

The ratio of labeled calcium in the skin to that in the skeleton was altered until, in this toxic water, the skin had an equal or greater concentration of labeled calcium. Significantly less of the absorbed calcium was transported to or exchanged with bone calcium. It appears that copper and zinc not only act externally to prevent normal ion absorption, but also act internally to alter the distribution pattern of the absorbed ions.

Cobalt and nickel. These intermediate-toxicity metals also reduced absorption of labeled calcium by about the same degree as the high-toxicity metals. Normal distribution ratios of labeled calcium to skin and skeleton were, however, retained in the presence of added calcium, barium, and potassium. The aforementioned increased labeled calcium distribution to the skin in the water with copper and zinc occurred this time only in the presence of added magnesium and strontium. The fish in the aquaria with added magnesium and strontium also suffered the highest mortalities.

In general, mortality was heavier in this greater concentration of the two intermediate-toxicity metals, directly because of the higher concentration, or because of additional competitive divalent ions provided by the cobalt, or simply because of a difference in the lethal effects of the different heavy metals. That their actions are different is indicated by a lack of increased calcium absorption in the presence of added potassium when the fish were in this intermediate-toxicity water.

Calcium exchange. Alterations in calcium exchange caused by the two groups of toxic ions differ enough to permit speculation that the mechanisms of action of these heavy metals are significantly different. Exchange of labeled calcium from the waters occurs mainly through the permeable surfaces of the gill tissues. Except where double the normal amount of dissolved calcium was present, the normal gill-to-skin distribution ratio of labeled calcium (about 1 to 1) was maintained in the water with added cobalt and nickel. With added copper and zinc, however, this distribution ratio was altered to nearly 2 to 1 in favor of labeled calcium in gill tissues. A differing action of these two groups of toxic ions affected both fish survival and the distribution of ions in the body. As far as the highly toxic copper and zinc ions are concerned, added alkaline earth cations and added salinity (provided by potassium) improved survival. Least mortality was observed in both the high- and intermediate toxicity solution when barium was the added ion. Magnesium, on the other hand, appeared to be somewhat antagonistic to calcium and did not reduce deaths in the presence of cobalt and nickel ions.

While the uptake of calcium from the water was profoundly affected by all of the added salts, ionic homeostasis appeared to be maintained in the presence of the supplemental alkaline earths. Likewise, the calcium-regulating mechanism malfunctioned in the presence of heavy metal ions, but this was somewhat ameliorated by the supplemental cations, which permitted survival of the brown trout in stressful water chemistries.

Henry A. Podoliak

COOPERATIVE STUDIES

Dr. William N. McFarland, physiologist at Cornell University, has participated in three cooperative studies at Cortland.

Dr. McFarland has, in one study, investigated the effect of long-term exposure of brook trout to continuous darkness, continuous illumination, and conditions simulating natural light on the amounts and ratios of the retinal visual pigments, rhodopsin and porphyropsin.

In another study he compared the levels and ratios of rhodopsin and porphyropsin in brook, brown, and rainbow trout and has analyzed for possible seasonal shifts in the amounts of these two visual pigments through monthly collections of retinae from the three species of trout for 18 months.

In a third study he conducted a histological examination of fish retinae. He could find no evidence of degeneration of retinae in brook trout held for over two years in either continuous darkness (except for brief exposures to infra-red light), continuous light, or simulated natural light.

Hugh A. Poston

WESTERN FISH NUTRITION LABORATORY

Cook, Washington
John E. Halver, Director

HIGHLIGHTS

Tiny coho salmon need 50 percent of diet as protein; then requirement drops with age to 40 percent at 12 weeks in 10° C. water. Yearling cohos need only 35-37 percent of diet as protein in 10° C. fresh water.

Fish protein concentrate is readily used as protein by cohos and rainbow trout.

No tumors occurred in cohos insulted with aflatoxin for one year and then converted and reared in sea water.

A vitamin test diet for Lahontan cutthroat trout was developed.

Urea nitrogen was less than 10 percent of total nitrogen excreted regardless of fish load or time of day.

Blood before and after gill passage shows that deamination occurs elsewhere than in gill tissue.

Gill Na^+, K^+-ATPase drops by July and fish will not convert to sea water. High activity occurs in March, April, and May and fish can convert. One can condition fish to convert by pretreatment with high salt diets.

Lahontan cutthroat trout need 4 percent salt in diet; higher protein diets are more efficient. Exercise seems mandatory for good growth and survival.

Some plant proteins are satisfactory for trout and salmon diets. Maintenance requirements were 25 cal/g/day in 15° C. water; thus, the most rapid growth is the most efficient growth for fish.

NUTRITION AND TOXICOLOGY

Protein requirements of coho salmon

Quantitative protein requirements for yearling coho salmon reared in 10° C. water at the laboratory were determined. Duplicate groups of cohos at initial average weight of 10 g were fed protein test diets containing 70 parts casein and 30 parts of gelatin to make 30, 35, 40, 45, 50, 55, and 60 percent protein. Almquist plots of performance index showed inflection points after two weeks on the test diet at about 35 percent of protein. The results were reinforced after 4, 6, and 8 weeks on test, indicating the tentative requirement of yearling cohos for protein to be about 35 percent of the ration in 10° C. water. No significant difference in protein, lipid, or ash deposited in the tissues was observed between fish on different diet treatments (Table 1).

Small cohos were similarly tested in 15° C. water at the Hagerman Field Station. Response of the coho fry was similar to that of rainbow trout and chinook salmon fed similar diets. Almquist plots of weight gains after 2 and 4 weeks on test indicated these small salmon needed approximately 50 percent protein in the diet, but 10 weeks on test indicated an inflection point at about 45 percent protein in the diet.

Another study with cohos from the same egg source and fed the same diets in 10° C. water at the laboratory suggested a need for

Table 1.--Carcass proximate analysis
Protein requirement--1969--coho salmon

% protein in diet	% protein in fish	% lipid in fish	% ash in fish	Totals	% H_2O
30	60.02	29.51	8.76	98.29	78.90
	61.63	29.28	8.84	99.75	78.60
35	61.87	30.28	8.66	100.81	77.96
	61.52	30.59	8.49	100.60	77.90
40	63.11	29.28	8.57	100.96	78.18
	62.91	30.80	8.64	102.35	77.98
45	63.19	29.29	8.28	100.76	78.22
	61.59	29.10	8.88	99.57	78.63
50	62.96	27.43	8.54	98.93	79.27
	63.88	27.75	9.01	100.64	79.15
55	63.44	28.44	8.42	100.30	78.36
	63.56	27.86	8.71	100.13	78.26
60	66.03	26.87	8.97	101.87	79.10
	65.95	26.20	8.78	100.93	78.75

approximately 45-50 percent of the diet as protein during the first 2-4 weeks of the feeding trial and then a subsequent drop in protein requirement to about 40-42 percent of the casein-gelatin mixture in the ration for maximum growth as fish grew to about 3 g in size. Figure 1 shows average weight gain vs. protein content of the diet for young coho salmon fed these diets for 4 and 10 weeks.

Protein sources for yearling coho salmon

Biological utilization of protein sources for yearling cohos was examined at the laboratory with Dr. Nose, guest scientist from the Freshwater Fisheries Research Laboratory, Tokyo, Japan. Fish protein concentrate, cottonseed flour, Torula yeast, egg albumin, casein-gelatin, soybean meal, and opaque-2 corn gluten were fed to 2-year-old cohos held in metabolism chambers in 10° C. water. Results from the metabolism chambers were related to growth response and diet efficiency observed during a 6 week feeding trial with yearling cohos fed these protein sources as half the protein component of the ration. Fish would not readily accept soybean meal or cottonseed flour protein diets; therefore, the feeding trial was designed with half the protein furnished by the test protein and half furnished by a casein-gelatin mixture. Amino acid patterns were determined on 16 diets and protei sources for this study. Metabolizable energy content of the diet and partition of the protein components between urinary, fecal, and branchial excretions were measured in cohos at Cook, Wash., and in large rainbow trout hel in metabolism chambers in 15° C. water at the Hagerman Field Station.

Feeding trials were designed to test result of the studies described above. In addition, after 4 weeks on test a cytoplasmic protein fraction extracted from soluble components removed during preparation of fish protein concentrate was added to one group of fish on each diet to enhance acceptability of the differe test diets. Then, 6 more weeks of feeding wer completed. Results of the feeding trials closel resembled results from the metabolism chamb analysis. Ethylene dichloride-prepared fish protein concentrate (Viobin) was the most readi used protein source, followed by casein-gelatin egg albumin, and Torula yeast; most poorly

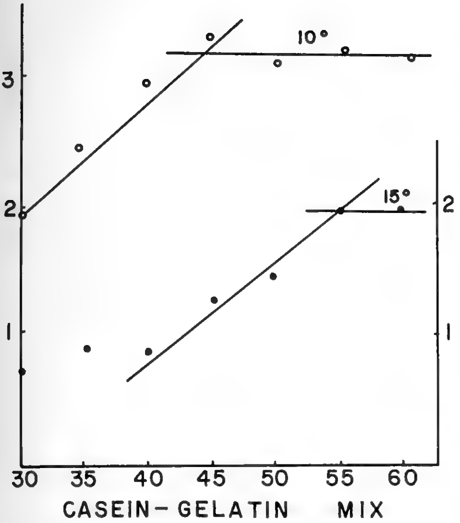


Figure 1.--Growth of coho salmon on different protein diets. Upper curve shows growth after 12 weeks on test in 10° C. water; protein requirement about 40% of diet (N x 6.25). Lower curve is plot of protein content vs. diet efficiency for small coho after 4 weeks in 15° C. water; protein requirement about 50% of diet (N x 6.25). All diets were approximately isocaloric between treatments.

used were cottonseed flour, soybean meal, and opaque-2 corn gluten for yearling coho salmon in 10° C. water. Small cohos in 15° C. water grew best when fish protein concentrate was the protein in the ration. Torula yeast was well used, followed by the casein-gelatin mixture, cottonseed flour, and soybean meal; poorly used for growth were egg albumin and opaque-2 corn gluten.

Cytoplasmic protein fraction made the cottonseed flour diet more acceptable but a negative correlation was obtained in those diets containing Torula yeast or soybean meal, and no correlation could be observed in diets containing opaque-2 corn gluten or the casein-gelatin mixture. Amino acid assay of components of CPF did not indicate adequate supplementary effect for the cottonseed flour protein diets to account for the observed increase in growth response. In fact, the increased growth could be directly correlated with the large amount of feed ingested and converted at the same efficiency as in that lot containing cottonseed flour protein without added CPF. No positive correlation between amino acid patterns assayed in FPC, TY, CSF, SBM, 02G or C-G protein component diets and the observed growth response could be obtained. Greatest feed efficiency of all protein sources tested was with the highly digestible casein-gelatin protein. Fish protein concentrate or Torula yeast will produce good growth in small cohos but the diet efficiency needs to be improved by either formulation or diet pretreatment to make the protein more available to these young fish. Results of the 6-week test of these components, with and without added CPF, show in Table 2.

Lahontan cutthroat trout test diet

Major effort at the Hagerman and Bowman Bay Field Stations resulted in one vitamin test diet which would rear Lahontan cutthroat trout for at least 16 weeks without the appearance of any nutritional deficiency syndrome. Studies included varying mineral and protein content of the ration in troughs, conical aquaria, and a tank that required the fish to swim continuously. Feeding results indicated that the test diet should contain about 4 percent mineral mix and about 50 percent protein. Better diet efficiency was observed with 70 percent protein in 15° C. water at Hagerman. Brine shrimp nauplii for tiny fish and a moist commercial pellet for much larger fish in the 10° C. water system at Bowman Bay worked well. Samples of fish obtained after 3 weeks on test showed fish fed brine shrimp nauplii had intestines filled with undigested chitinous exoskeletons, however, and soon thereafter growth ceased unless these fish were shifted to moist pellets or casein-gelatin rations.

Fish fed the laboratory complete test diet grew well but often had extended stomachs when reared in still or slowly moving water systems. In contrast, those fish reared in nose-cone aquaria or in round tanks where violent exercise was a continuous demand showed good growth, diet conversion, and diet efficiency. In tests

Table 2.--Protein sources for coho at 15°C

Protein	Number of fish	Average weight	Feed used	Diet efficiency
Fish Protein Concentrate	199	2.37	309	0.81
Torula Yeast	200	2.25	133	1.03
Torula Yeast plus CPF	200	2.05	123	1.04
Cottonseed Flour	199	1.77	111	0.96
Cottonseed Flour plus CPF	198	2.26	156	0.94
Soybean Meal	189	1.70	90	0.79
Soybean Meal plus CPF	191	1.50	70	0.79
Opaque-2 Gluten	198	0.61	69	0.17
Opaque-2 Gluten plus CPF	200	0.67	68	0.31
Egg Albumin	196	1.13	142	0.48
Casein-Gelatin	193	1.83	118	1.53

in a deep square tank with rounded corners, water was jetted into the center to cause circulation of the water mass. Small Lahontan cutthroat trout reared under these conditions grew well, with about 85 percent survival through an initial 20-week feeding trial in 15° C. water at Hagerman.

Chronic aflatoxicosis in coho salmon

Cohos fed diets containing 20 ppm aflatoxin B_1 failed to show any hepatoma after one year on test in fresh water. These fish were then moved to the Bowman Bay Field Station and adapted to sea water. After 18 months in sea water, all surviving fish were examined by necropsy, and liver tissue preserved for subsequent histopathology. No hepatomatous tissue was observed in any fish examined at necropsy, and no hyperplastic or neoplastic tissue was detected in any of the slides examined microscopically. Coho are remarkably refractive to chronic insult with aflatoxin B_1.

John E. Halver

ENZYMOLOGY

Determination of ammonia and urea in trough water

The ammonia concentration in water from troughs holding usual loads of fish can be determined routinely by some variation of the indophenol method, such as that of Muramatsu, Agr. Biol. Chem., 31:301 (1967). Such direct colorimetric determination would be subject to interference from color produced from other substances, but the interference would probably be minimal for routine analyses. Care must be observed in collecting proper blank samples because it has been found that water flowing into troughs sometimes contains considerable and variable (with time) color-producing material.

The concurrent determination of ammonia and urea under similar conditions presents problems that have not been completely solved. The required addition of urease to hydrolyze the usual low concentration of urea (near the limit of sensitivity of the method) introduces color-producing materials that create an intolerable blank situation for revealing urea-ammonia by differences. Various methods used to concentrate the urea-ammonia seemed only to complicate the urease interference. Reliable results for relative amounts of excreted urea- and ammonia-nitrogen were obtained by treating trough intake and effluent water in microdiffusion dishes (modified Conway). Urease was even added (inactivated) to the dishes used for the determination of preformed ammonia by adding it after the alkali was added to release the ammonia. Alkali was added to the dishes for total ammonia after 30 minutes of urease-hydrolysis. The diffused ammonia was determined by the indophenol method.

The above procedure shows that the amount of metabolic urea-nitrogen was always relatively small compared to the ammonia-nitrogen. Although the absolute amount of urea-nitrogen was not accurately determined, it was less than 10 percent of the excreted ammonia-nitrogen determined under identical conditions. This small percentage of the total was shown to be in trough water regardless of the load of fingerling chinook salmon (1.8 to 15.4 lbs/gal inflow/min) or time of day of sampling (4 hours of continuous sampling in the middle of the day or the night). There was some indication of an expected increase in ammonia concentration during the day due to increased activity of the fish.

GOT, GPT, GDH levels in liver, gill, and serum

Spectrophotometric clinical procedures (Sigma 410-UV) for determining glutamic-oxalacetic transaminase (GOT) and glutamic-pyruvic transaminase (GPT) in serum were modified for use with fish tissues. It soon became evident that light absorption at 410 mμ decrease in a mixture of the dialyzed tissue homogenates, a-ketoglutarate, DPNH, and ammonia even when malic dehydrogenase (MDH) or lactic dehydrogenase (LDH) were not added as indicators for the GOT or GPT reactions, respectively. This would most logically seem to be due to glutamic dehydrogenase (GDH) activity (Figure 2). Because a combination of

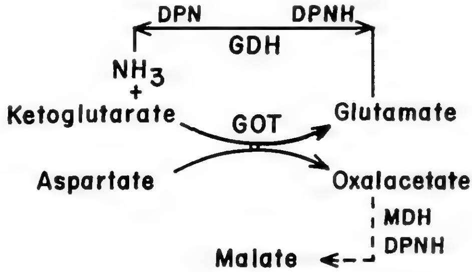


Figure 2.--Glutamic-oxalacetic transaminase and glutamic dehydrogenase reactions.

GDH with GOT (GPT) activity is often postulated as the pathway for deamination of amino acids, it was decided to determine GDH along with GOT (aspartic acid as substrate) or GPT (alanine as substrate) by obtaining three separate reaction rates for each transaminase, as follows:

A. All reagents present for total GDH and GOT (GPT).

B. MDH (LDH) in ammonium sulfate solution omitted for endogenous material.

C. MDH (LDH) omitted, ammonium sulfate added for GDH and endogenous material.

C - B = GDH activity

A - (C - B) = GOT (GPT) acitivty

The calculations gave two GDH values for each tissue--one from the GOT and one from the GPT determination. In most cases these two values were fairly similar except for serum, in which case the GPT-determined values seemed more reliable and were therefore used in calculating all GOT as well as GPT values. (The GOT-determined GDH values of serum were often negative, due, it is thought, to a build-up of glutamate from the relatively high GOT activity and consequent reversal of the GDH-promoted reaction.)

Average GOT, GPT, and GDH values found for the gill, liver, and serum of rainbow trout and of coho salmon are shown in Table 3. All of the trout were from one population, but the starved group had not been fed for at least 4 weeks. The GPT values for coho gill and liver were significantly higher ($p = 0.01$) than the respective values for rainbow trout. Also, the GOT value for coho serum was significantly lower than for trout serum. Although there were considerable differences in the GPT and in the GDH values for sera of the two species, the significances were low. The differences may not be species differences because there were some uncontrolled factors. Again, big differences in certain values (e.g., gill GPT and serum GPT and GOT) for fed compared to starved rainbow trout were of little significance because of wide variations among individuals.

The level of fish serum GOT was about 10-fold higher than that of normal human serum GOT. However, on a per-gram protein basis, all the fish serum activities were lower than the activities in the other fish tissues.

Deamination routes

It has been shown above that the fish tissues contained measurable transaminase and glutamic dehydrogenase activity which may function for the main route of *in vivo* deamination of amino acids. Analyses for ammonia concentration in blood removed from live fish via the bulbus arteriosus and the artery in the roof of the mouth indicated a decrease as blood passes through the gills. This probably precludes the gill as being the main tissue for deamination. When certain amino acids were added to dialyzed tissue homogenates with other reagents shown in Table 4, ammonia was produced.

C. Bradford Croston

Table 3.--Average transaminase and deaminase activities in fish tissues

	GOT	GPT	GDH
		ΔOD/min/g Tissue	
Gill			
Rainbow trout	7.8(10)*	0.8(10)	6.0(10)
Starved rainbow trout	6.0(10)	2.0(10)	4.9(10)
Coho salmon	10.5(11)	3.4(11)	4.6(11)
		ΔOD/min/g Tissue	
Liver			
Rainbow trout	26.3(10)	10.4(10)	8.2(10)
Starved rainbow trout	29.1(9)	13.3(10)	11.9(8)
Coho salmon	26.5(11)	20.1(11)	6.6(11)
		SGOT Units (Sigma)	
Blood serum			
Rainbow trout	510.0(8)	22.3(9)	72.0(9)
Starved rainbow trout	246.0(8)	43.6(8)	54.6(8)
Coho salmon	231.0(5)	30.5(5)	23.7(4)

*Numbers in parentheses are numbers of individual fish.

Table 4.--Reaction mixture for ammonia formation from amino acids

Volume (ml)*	Reagent
0.06	Amino acid solution (0.4^{M}DL-form or 0.2M L-form)
0.12	Tissue homogenate (in pH 7.5 phosphate buffer)
0.02	a-Ketoglutarate solution (0.1 M)
0.02	DPN solution (2 mg/ml)
0.04	Sodium arsenite solution (0.033 M)
0.02	Pyridoxal phosphate solution (200 μg/ml)

*Volumes added to each modified Conway microdiffusion dish.

MINERAL METABOLISM

Gill ATPase activity in coho salmon

Seasonal changes in gill ATPase activity of cohos indicate that a relation exists between the Na^+, K^+-stimulated activity and seaward migratory movements of juvenile fish. Constant monitoring of activity from February to October has shown that this activity in microsomal particles from gill tissue of yearling coho maintained at a constant 10° C. (50° F.) increased from about 13 μmoles ATP hydrolyzed/mg/protein/hr to 26 during the last two weeks of March. Activity remained at this level until the first of July, at which time it began to decrease, reaching a value of about 18 μmoles by the end of the month and remaining at that level through the first of October. This information is given in line one of Table 5. The increased Na^+, K^+-ATPase activity appears at a time when these salmon normally begin migrating to sea and may be a biochemical manifestation of their readiness to accept sea water. This activity may also have played a role in results obtained by Baggerman (J. Fish. Res. Bd., 17:295(1960)) in which young coho salmon showed a preference for fresh water at the beginning of March, but for salt water at the end of the month and during April and May. In July the test animals again selected fresh water, which may bear a direct relation to the July decrease in Na^+, K^+-ATPase activity. Noble (Proc. N. W. Fish. Cult. Conf., pp. 48-51(1958)) reported that coho from rearing ponds in Minter Creek, Wash., in December did not migrate until April. Coho placed in the creek in July did not show an appreciable migration.

The release of hatchery reared fish early enough in the spring to complete seaward migration by July may be an important factor in determining the total number making transition into sea water. Additional support for the suggestion that a seasonal elevation in gill Na^+, K^+-ATPase activity might be related to salt water selection and adaption is the observed elevation of this activity when coho salmon adjust to a salt water environment. Values for this activity increase 3- to 4-fold over the fresh water levels (Table 5, Line 5).

The enzyme responsible for this ATPase activity has a role in transporting Na^+ across membranes and it would be expected that greater activity is required when the animal resides in sea water and must eliminate salt into a hypertonic environment.

The basic Mg^{2+}-ATPase activity decreased from about 42 μmoles P_i released/mg pr/hr in fresh water to 21 when the fish became fully adapted (Table 5, Lines 1 and 5). The reason for this decrease is not fully understood at this time but a similar decrease was detected when fish were fed a diet containing 12 percent (dry weight) added NaCl (Table 5, Line 2). Feeding

Table 5.--Summary of ATPase activity in gill microsomes from coho salmon

Conditions	ATPase activity (μmoles P_i released/mg pr/hr)					
	February to mid March		Mid March to mid July		July to October	
	Mg^{2+}	Na^+, K^+	Mg^{2+}	Na^+, K^+	Mg^{2+}	Na^+, K^+
1. Complete test diet (CTD)	42	13	42	26	42	18
2. CTD + NaCl (12%, dry wt)	26	17	26	33	--	--
3. CTD (exercise)	--	--	58	26	--	--
4. Hatchery raised	--	--	60	24	--	--
5. Salt water adapted	--	--	21	93	--	--

NaCl also caused a moderate elevation of the Na^+, K^+-ATPase activity.

Cohos obtained from the Willard National Fish Hatchery showed higher Mg^{2+}-ATPase activity (Table 5, Line 4) than our laboratory fish held in troughs. We therefore took samples from laboratory fish in circular tanks and found a correspondingly elevated Mg^{2+}-activity (Table 5, Line 3). Since the only obvious difference between the laboratory fish held in troughs and circular tanks was the amount of exercise required to maintain position we surmised that exercise might be a factor which influence observed activities.

Table 6 shows that the seasonal changes in Na^+, K^+-ATPase activity were relatively independent of the size of the fish. Activities in fish of 11.0 to 11.9, 12.0 to 12.9, and 14.0 to 14.9 cm groups measures 12, 14, and 15 during February 12 to March 15, while fish in the same size groupings had activities of 22, 25, and 28 during March 16 to July 31. Likewise, overlappings in the size groups from March 16 to July 31 and from August 1 to October 2 showed that the season and not the size was the determining factor in Na^+, K^+-ATPase activity. An exception was seen in the large fish (18.0 to 23.9 cm), which seemed to maintain an elevated activity after July 31. However, these fish were considerably larger than normal migrants.

Data in Table 7 show how the Na^+, K^+-stimulated ATPace activity increased and the Mg^{2+}-dependent activity decreased when coho salmon were placed in salt water (35 ppt., Instant Ocean salts). Both activities reached maximal change after 30-35 days, suggesting that cohos are not completely adapted until salt water residence has been established for this length of time. The final values in the table were obtained from 4 coho adapted to natural sea water (31 ppt) at Bowman Bay.

We have attempted to show the minimum length of time in salt water required to produce changes in gill ATPase activities. In these studies we have followed the changes in individual fish by surgically removing samples of the gill filaments at various salt water exposure times. Under anesthesia (MS-222) filaments from one or two arches of fresh water coho were removed with scissors and used for ATPase determinations. The fish were allowed to recover completely (4 hours) and were then placed in full strength sea water (Instant Ocean salts, 35 ppt) for a desired length of time. The fish were again anesthetized and filaments removed for study. Changes in ATPase activity were detected only after 3 to 4 days in salt water.

Table 6.--Relationship between size of fish and season of the year on Na^+, K^+-activated ATPase activity of coho salmon gill microsomes*

Fork length (cm)	Na^+, K^+-activated ATPase activity (μmoles P_i released/mg pr/hr) February 12 to March 15	March 16 to July 31	August 1 to October 2
11.0 - 11.9	12 (2)**	22 (2)	--
12.0 - 12.9	14 (6)	25 (3)	--
13.0 - 13.9	--	26 (8)	--
14.0 - 14.9	15 (2)	28 (6)	12 (1)
15.0 - 15.9	--	23 (3)	16 (2)
16.0 - 16.9	--	23 (1)	17 (6)
17.0 - 17.9	--	--	16 (1)
18.0 - 23.9	--	--	22 (5)

*Experimental conditions listed in Table 5.

**Number of fish in parentheses.

Table 7.--Changes in ATPase activities upon exposture of coho salmon to salt water

Days in salt water	ATPase activity (μmoles P_i released/mg pr/hr)[1] Mg^{2+}	Na^+, K^+
0	42	26
7	42	40
15	30	59
22	28	70
29	23	91
36	22	95
39	19	93
46	22	102
58	20	84
63	20	102
67	25	82
77	18	90
70[2]	22	82

[1]Values given are averages of at least two fish.

[2]These fish (4) were held in natural sea water at the Bowman Bay Field Station for 2 months.

ATPase purification

Partial purification of the enzymes containing ATPase activities has been achieved by sucrose density gradient centrifugation. In the most successful preparation thus far the Mg^{2+}-ATPase activity was increased from 20 moles P_i released/mg pr/hr to 69, a 3.5-fold purification. In the same fraction the Na^+, K^+-ATPase activity was increased from 56 to 258 moles P_i mg pr/hr, a 4.6-fold purification. This magnitude of Na^+, K^+-ATPase activity has been achieved in tissues of other animals only after extensive purification procedures. It might, therefore, be possible to obtain a purified enzyme from salt water-adapted fish having activities exceeding those currently being studied in other animals.

Waldo S. Zaugg

PHYSIOLOGY

Salmon Into Sea study

Cooperative use of the Bowman Bay Station by this laboratory, Bureau of Commercial Fisheries Biological and Technological Laboratories, and the International Pacific Salmon Commission has been quite successful. Additional space is being developed for new aquaria to partially replace and double the holding capacity of the experimental hatchery.

High water temperatures in the experimental hatchery were corrected this past summer with the installation of a heat exchanger and preliminary tests indicate a sand filter and ultraviolet light system have corrected algal and disease problems. Vibrio in the sea water system necessitated the early termination of protein studies with yearling coho salmon in a sea water environment.

Outside facilities at the station were used extensively by biology classes for demonstration purposes of marine life. An undetermined number of individuals toured the station in conjunction with their visit to the adjacent park area which ranks second in population usage of the entire Washington State Park system.

Lahontan cutthroat trout tests

Tests with Lahontan cutthroat in 1968 indicated that greater efficiency and growth were obtained with a commercially prepared ration as the fish became older. The results of a 14-week feeding trial with yearling Lahontan trout are summarized in Table 8.

Table 8.--Growth data: 14 week trial with yearling Lahontan cutthroat

	Diet	% Weight gain	% Survival	Gain/g dry diet fed	Hematocrit ± S.E.
Fresh water	8-S*	173	82	.65	50 ± 1
	285**	198	97	.86	48 ± 2
5% sea water	8-S	240	67	1.04	44 ± 1
	285	195	85	.92	46 ± 1
10% sea water	8-A	211	68	.89	44 ± 2
	285	192	86	.82	43 ± 2

*Diet 8-S contains 8% minerals.

**Diet 285 contains 14% minerals.

Fish were fed either the Oregon Moist Pellet, containing approximately 14 percent dry weight as salts, or a test diet with 8 percent dry weight as minerals. Growth in fish fed the OMP was nearly the same in the 3 water environments. It surpassed the growth from Diet 8-S-fed fish in fresh water but was less in a brackish water environment.

New test diets were designed to measure response of coastal cutthroat and Lahontan cutthroat trout to various mineral levels in the diet.

Initial feeding fish were challenged with diets containing 0, 1, 4, and 8 percent salt in the complete test diet. These were compared with others receiving Oregon Moist Pellet, fish meal concentrate, and cytoplasmic protein concentrate. Two groups from the same egg source were divided between (1) Hagerman Field Station to be held in circular glass aquaria or still water troughs in 15° C. hard water systems (approximately 220 ppm total dissolved solids) and (2) Bowman Bay Field Station to be held in fresh water (170-190 ppm T.D.S.) or 10 percent sea water systems (approximately 3300 ppm T.D.S.). As a comparison, total dissolved solids of approximately 400 ppm and 5000 ppm, respectively, are found at Summit Lake and Pyramid Lake, the two primary sources of Lahontan broodstock.

Fish at Bowman Bay were held in "nose-cone" type aquaria, modified from airplane wing tanks.

Growth of initial feeding coastal cutthroat trout at Bowman Bay was lower at the end of 6 weeks in 10 percent sea water than in a fresh water environment (Table 9). Survival was generally higher in fresh water-raised fish, a notable exception being with fish fed with no mineral supplement to the diet. The largest weight gain was found in a test diet in which fish meal provided the protein component (Diet 284). Little difference was observed in proximate analyses of carcasses at end of feeding trial (Table 10).

At Bowman Bay Lahontan sac-fry were started on test diets at swim-up. Water temperature was 13° $\pm$ 1° C. throughout the 24 week experiment. Growth trends shown in Table 11 are raw data and have not been subjected to statistical analysis. Many physical requirements have yet to be defined to ensure maximum survival, but it would appear that the present laboratory test diet is satisfactory for preliminare diet testing with the Lahontan trout. There appears to be a shift in the physiological status of these fish somewhere between 10 and 14 weeks that affects the rate of growth in a 10 percent sea water system. This change is affected by the concentration of minerals in the diet being fed.

Amino acid patterns in fish tissue

Amino acid contents of whole carcass tissue from yearling rainbow trout, coho salmon, and chinook salmon were measured for reference values for future work. All animals were maintained on the laboratory complete test diet for one year in 10° C. water before analysis of tissue (Table 12).

Clarence L. Johnson

AVAILABILITY AND UTILIZATION

Metabolizable energy of feed materials

Availability and utilization studies this year were concerned with the testing of feed materials for metabolizable energy (ME) content, the indirect measurement of heat production, and the evaluation of several materials as protein sources for coho salmon.

The ME content and the digestibility of the following were measured using large rainbow trout in metabolism chambers: Torula yeast, egg albumin, fish protein concentrate, soybean meal, corn gluten, cottonseed meal, dry skim milk, blood meal, wheat gluten, poultry by-products meal, white fish meal, field pea meal, dried whey, and brewer's yeast. These indicated that some plant proteins are suitable for inclusion in fish feeds. However, these plant proteins failed to produce satisfactory growth in feeding trials because the fish refused to eat adequate amounts of diets when a substantial part of the protein was from plant sources. It appears

Table 9.--Growth data: Six week feeding trial of coastal cutthroat trout at Bowman Bay

Diet*	% Weight gain		% Survival	
	Fresh water	10% Sea water	Fresh water	10% Sea water
8	188	175	91.7	91.3
8-S	143	--	91.0	--
289	121	118	85.0	96.3
290	128	135	87.9	90.4
291	168	158	95.8	90.8
292	187	158	100.0	92.5
284	245	202	92.1	92.1
285	166	160	85.0	81.7

*Diet Legend: 8: laboratory complete test diet (CTD); 8-S: CTD with two times standard mineral packet; 289: CTD without mineral packet; 290: CTD with one-four times mineral packet; 291: CTD with 1% cytoplasmic protein concentrate from fish meal; 292: CTD with 1% isopropyl alcohol washed cytoplasmic protein concentrate; 284: CTD with commercially prepared fish meal substituted as protein component; 285: commercially prepared fish food.

Table 10.--Proximate analysis of terminal samples of coastal cutthroat feeding at Bowman Bay

Diet*		% Protein	% Lipid	% Ash	Total	% Water
8	Fresh water	74.0	17.7	9.5	101.2	85.6
	10% Sea water	74.2	17.0	9.5	100.7	85.7
8-S	Fresh water	72.3	19.5	8.7	100.5	84.8
289	Fresh water	71.7	19.7	8.9	100.3	86.8
	10% Sea water	72.2	20.9	8.4	101.5	86.7
290	Fresh water	74.9	18.6	8.9	102.4	86.1
	10% Sea water	73.5	18.5	8.5	100.5	85.5
291	Fresh water	74.4	16.6	9.7	100.7	85.5
	10% Sea water	75.4	16.3	9.3	100.0	85.5
292	Fresh water	73.8	17.6	9.6	100.0	86.2
	10% Sea water	75.2	18.4	9.5	103.1	85.5
284	Fresh water	75.8	14.6	10.4	100.8	84.6
	10% Sea water	75.0	16.5	10.3	101.8	84.9
285	Fresh water	74.1	18.4	9.0	101.6	85.8
	10% Sea water	74.2	19.9	9.3	103.4	86.0

*See Diet Legend, Table 9.

Table 11.--Growth in Lahontan cutthroat trout at Bowman Bay*

Diet		6 Weeks		10 Weeks		14 Weeks		24 Weeks	
		Avg[1] weight	%[2] survival	Avg weight	% survival	Avg weight	% survival	Avg weight	% survival
8-S	Fresh water	.34	87	.61	84	.96	47	2.53	44
	10% Sea water	.30	67	.51	64	1.01	37	2.96	26
8	Fresh water	.35	79	.66	75	1.15	34	3.57	31
	10% Sea water	.31	72	.58	69	1.35	31	4.16	27
289	Fresh water	.28	100	.44	79	..54	78	.87	45
	10% Sea water	.24	85	.36	83	.53	56	.77	42
290	Fresh water	.33	71	.51	67	.77	53	1.43	46
	10% Sea water	.28	84	.46	82	.71	50	1.07	45
291	Fresh water	.46	82	.63	77	.96	56	2.86	48
	10% Sea water	.40	88	.61	86	.98	56	3.13	23
292	Fresh water	.38	85	.66	80	1.07	45	3.04	39
	10% Sea water	.33	83	.57	82	.96	44	2.66	36
284	Fresh water	.55	63	1.01	56	1.47	46	3.90	38
	10% Sea water	.24	62	.79	44	1.37	37	3.75	25
285	Fresh water	.58	56	1.10	52	2.06	52	5.92	49
	10% Sea water	.49	39	1.06	38	2.30	33	6.52	31

*NOTE: Observe shift in growth responses between 10 and 14 weeks in the fresh vs. 10% sea water systems. (See legend Table 10 for diets.)

[1]Average initial weight, 0.14 g.

[2]Includes loss from all causes. Considerable cannibalism occurred in some groups.

Table 12.--Amino acid analysis of whole carcass (minus G.I. tract)

Analysis	Rainbow trout		Chinook salmon		Coho salmon	
	% Dry Wt	% Nitrogen	% Dry Wt	% Nitrogen	% Dry Wt	% Nitrogen
Lysine	5.06	8.34	5.67	9.04	5.65	8.67
Histidine	1.86	4.33	1.92	4.32	1.99	4.32
Ammonia	0.88	6.19	0.95	6.51	1.01	6.65
Arginine	4.06	11.21	4.16	11.13	4.08	10.49
Aspartic Acid	6.55	5.92	6.71	5.88	7.01	5.91
Threonine	2.57	2.59	3.06	3.00	3.18	2.99
Serine	2.96	3.40	3.02	3.35	3.15	3.36
Glutamic Acid	9.12	7.46	10.00	7.92	10.44	7.95
Proline	2.83	2.96	3.16	3.20	3.11	3.03
Glycine	5.36	8.60	5.10	7.91	5.58	8.33
Alanine	4.15	5.61	4.24	5.54	4.50	5.65
1/2 Cystine	0.38	0.38	0.42	0.40	0.44	0.41
Valine	2.90	2.98	2.98	2.97	3.10	2.97
Methionine	2.10	1.69	2.15	1.68	2.19	1.64
Isoleucine	2.37	2.17	2.54	2.26	2.57	2.20
Leucine	4.82	4.42	5.10	4.53	5.14	4.40
Tyrosine	2.07	1.37	2.19	1.41	2.31	1.43
Phenylalanine	2.49	1.82	2.63	1.86	2.70	1.83
Taurine	0.14	0.13	0.18	0.16	0.21	0.19
Total %	62.67	81.59	66.16	83.07	68.37	82.43
Dry Wt on Column		891.8μg		1047.0μg		927.0μg
Nitrogen on Column		103.8μg		125.8μg		115.8μg
Protein Analysis by Macro-Kjeldahl		72.19		74.26		78.62
% Moisture		78.0% H_2O		80.96% H_2O		81.06% H_2O

that properly balanced diets containing mostly plant protein can be used if the diets can be made acceptable to the fish.

Indirect measurement of heat production

Groups of fish were fed diets of known ME content at approximately 100 percent, 80 percent, 60 percent, and 40 percent of voluntary food intake. The fish were weighed biweekly and were sacrificed at 12 weeks. The carcasses were analyzed for deposited protein, and calories and efficiency of food utilization was calculated. The energy lost as heat was calculated by the formula: Heat Production = ME - Stored Energy.

The results indicate a maintenance requirement of about 2500 cal/100 g body weight/day for small coho salmon in 15° C. water. Body maintenance required about 75 percent of the ME fed at the lowest level and only about 40 percent of the ME fed at the highest level. These data indicate that with fish, as with other animals, the most rapid growth is the most efficient growth.

Urine flow

Tests were conducted to measure variations in urine flow during confinement in metabolism chambers. Fish were fed two hours before confinement, then urine flow was collected, removed, and weighed at 0800 and 1630 hours daily. Average weight of 5 fish on test was about 400 g each. Samples were collected for 10 days on test. Average urine flow was about 15 percent of body weight/day, approximately evenly divided on an hourly basis, day or night.

Robert R. Smith

HISTOPATHOLOGY

Cutthroat trout mortalities

Lahontan cutthroat fry reared at the Hagerman Field Station began dying approximately 3 weeks after first feeding. Moribund fish from each diet group were autopsied on site (22 days on test) but no consistent pathological entity could be detected. Some fish reported to have swollen and excessively red gills died before samples could be preserved. Moribund fish examined later at necropsy and preserved for examination failed to show serious gill anomalies and no case of gill hemorrhage could be verified. Microscopically, some gills appeared slightly hyperemic and had slight hyperplasia of gill epithelium, but the most conspicuous finding was the general occurrence of short, widely-spaced gill lamellae. This suggests a possible hereditary anomaly which might have endowed a certain percentage, possibly one-fourth, of the population with inadequate respiratory epithelium for a successful and independent life. Smears from skin and gills were negative for bacteria after Gram staining but a few fish had long Gram-positive bacilli in small clumps in the posterior intestine. Some moribund fish had bloated stomachs and/or intestines (Figure 3), particularly those in rectangular troughs and usually the larger fish of a given trough, but bloating also occurred in several large control fish fed only complete test diet (CTD): it therefore may have been due to engorgement. More rapidly growing fish reared in 5 gallon plastic cylinders in which water circulation kept feed agitated and thus encouraged feeding activity also may have encouraged engorgement. Fish fed brine shrimp larvae in the cylindrical tanks began dying on Day 18. Histological examination of these moribund fish showed numerous undigested larval exoskeletons in the hind gut which appeared to cause bowel obstruction that may have resulted in the death of some of these fish (Figure 4). Cutthroat fingerlings 7 weeks on test and sampled when very moribund sometimes had skeletal muscle atrophy with inflammation and occasionally had petechial hemorrhages (Figure 5). Five control fish each, from the 3 and 7 week samples, were free from intestinal bacteria and had no muscle atrophy. Since muscular atrophy of affected fish involved myotomes both anterior and posterior to the dorsal fin the muscle anomalies conform to the pattern reported for viral hemorrhagic septicemia (VHS). However, evidence of hemorrhage was so minimal in the cutthroats examined as to render unlikely the possibility of these fish being infected with VHS. Several moribund fish from rectangular tanks were infected with water mold, probably Achlya or Saprolegnia. Mold hyphae were conspicuous in both hematoxylin and erosin- and in Giemsa-stained sections of kidney, ovary, pancreas, esophagus, intestine, muscle, and thymus gland of moribund fish. When mold infected the thymus, it was sometimes hemorrhagic (Figure 6).

Cohos refractory to aflatoxin B_1

Coho salmon fed CTD plus 20 ppb aflatoxin B_1 for 12 months in 15° C. spring water and then transferred to running sea water at 12° C. for 18 months failed to develop a single microhepatoma nodule.

Channel catfish on lipid diets

Sections cut at 6 microns from 150 channel catfish livers were stained with hematoxylin and eosin, Best's carmine, and with periodic acid Schiff (PAS) counter stained with fast green. Best's carmine and PAS are specific for tissue glycogen. Representative liver sections from each diet group were digested with diastase for 20 minutes at room temperature after which PAS positive materials were almost entirely lacking, proving the bulk of the PAS positive material to be glycogen. Experimental fish and controls all contained liver glycogen in amounts ranging from sparse to abundant. The kind of lipid fed did not appear to alter the presence or the amount of glycogen in the cytoplasm of liver parenchyma cells. Insignificant amounts of ceroid in portal areas and of focal necrosis in liver parenchyma were noted in several livers but were not limited to those representative of any particular diet (Figure 7).

Histology of mouth and pharynx

A detailed atlas of histology of the salmonid mouth and pharynx is in preparation. Taste may be involved, along with smell, in the "homing" migrations of salmons, steelheads and other anadromous species. Taste buds are distributed on lips, roof and floor of mouth, tongue, and pharynx back as far as the beginning of the esophagus. Salmonid taste buds are similar to those of higher vertebrates in histological detail. They are pale ovoid structures within a darker-staining stratified squamous epithelium and consist mainly of spindle-shaped supporting cells mingled with slender neuroepithelial cells, each terminating in a short hair-like structure in the taste pore at the surface.

Teeth are located not only on upper and lower jaws but on the roof of the mouth (on palatine bones, bilaterally; on the vomer bone, medially) and on the tongue. Additional teeth occur in the posterior portion of the pharynx--the pharyngeal teeth (Figure 8). All salmonid teeth are simple conical or recurved unicuspid in type and are used for grasping and holding food when necessary. Long recurved teeth occur in positions corresponding to those of canine teeth in higher vertebrates, on either jaw, but similar recurved teeth may also occur on the tip of the tongue (Figure 9). Salmonid teeth appear to develop after the manner of placoid scales characteristic of sharks (Chondrichthyes). This involves tooth buds consisting of inner pulp with dentine-forming odontoblasts derived from dermal mesenchyme plus an outer cup-shaped enamel organ lined with enamel-forming ameloblasts (Figure 10). Enamel organs are derived from ectoderm--the stratified squamous epithelial mucous membrane of the mouth. The linings of mouth and pharynx are amply supplied with mucous-secreting epithelial cells whose secretions protect the delicate membranes and lubricate food materials for easy swallowing.

Histology of 18-year-old Bunny Lake brook trout

A total of 104 tissue slides of 18-year-old Bunny Lake brook trout and 3- to 4-year-old Cloverleaf Lake brook trout were examined for the Sierra Nevada Aquatic Research Laboratory to compare senile changes, if any, of the 18-year fish. The trout were reared from fry planted in Bunny Lake located above the 11,000 foot level in the Sierra Nevada of California. Cloverleaf Lake lies at an altitude of approximately 1000 feet and is eutrophic as compared with the highly oligotrophic Bunny Lake. Very few senile changes could be found in the 18-year-old trout; those noted include thickened basement membranes in renal glomeruli, hyalinization of some pancreatic arteries, slight skeletal muscular degeneration, and a greater number of atretic and degenerating ova.

Figure 3.--Sagittal section through cutthroat trout fry showing bloated stomach. Fish fed synthetic diet including 50% protein but no added minerals for seven weeks. Hematoxylin and eosin (H and E). X 44.

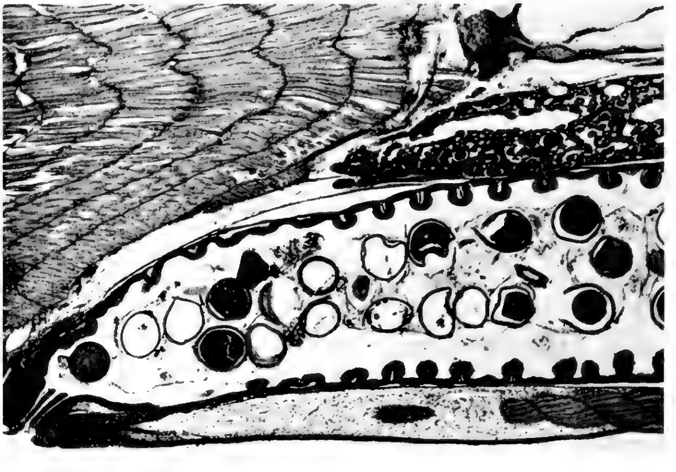


Figure 4.--Section of cutthroat trout fed brine shrimp nauplii for three weeks. Note hind gut partially obstructed by undigested exoskeletal remains. H and E. X 70.

Figure 5.--Section of cutthroat trout skeletal muscle showing muscle atrophy and inflammation (hyperemia). Diet included 4% mineral and 70% protein. H and E. X 175.

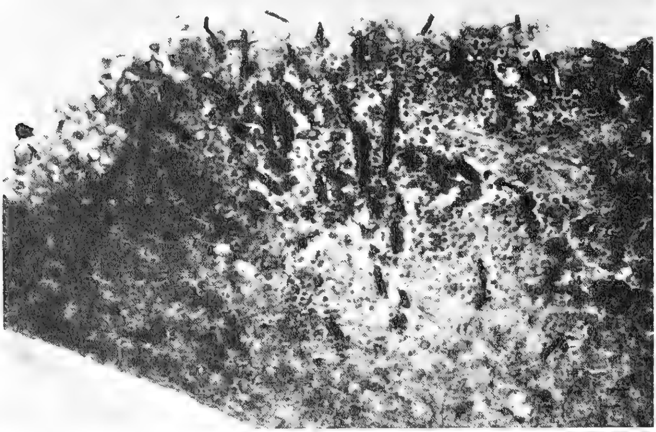


Figure 6.--Hemorrhagic necrosis in focal fungal infection of cutthroat trout thymus gland. Black strands are sectioned portions of mold hyphae, most of which are surrounded by hemorrhaged blood. Diet was brine shrimp nauplii.

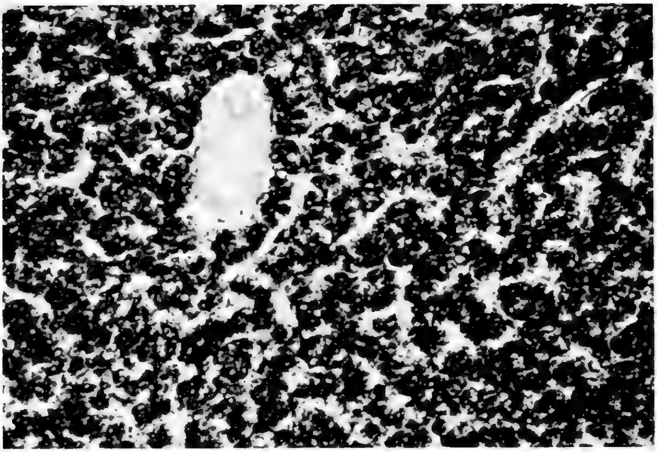


Figure 7.--Section of channel catfish liver showing heavy deposits of cytoplasmic glycogen in liver cells. Periodic acid Schiff counterstained with fast green. Diet included 20% corn oil. X 450.

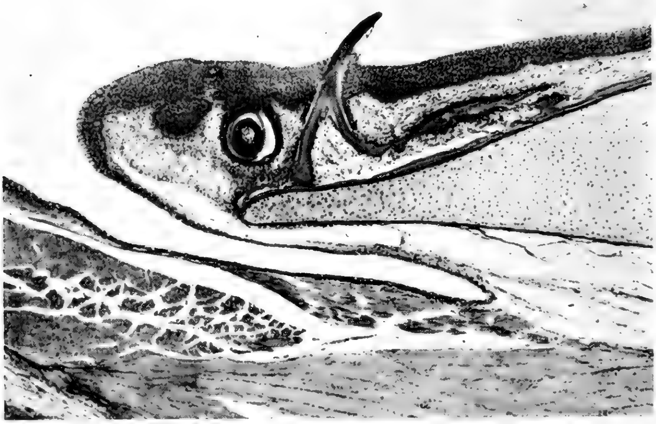


Figure 8.--Sagittal section through tongue of cutthroat trout showing a strong, recurved lingual tooth near tip of tongue. Note bony anchorextending caudal from posterior root of tooth. An oval-shaped unerrupted or embryonic tooth lies adjacent to the anterior root of the recurved tooth. Diet included 4% added mineral and 50% protein. H and E. X 110.

Figure 9.--Section through pharynx of cutthroat trout showing two pharyngeal teeth deep in the epidermis. Several taste buds occur at the surface above the larger tooth. Diet same as in the above illustration. H and E. X 450.

Figure 10.--Section through twotaste buds in mouth of adult 12-year-old brook trout. A blood capillary can be seen in the dermal papilla below the bud on the right. H and E. X 500.

Cloverleaf Lake fish had heavily vacuolated liver cell cytoplasm characteristic of well fed fish, while those from Bunny Lake were typical of poorly fed or starved fish, with almost no cytoplasmic vacuoles in liver cells. This obviously indicates a comparative absence of stored liver glycogen in the 18-year-olds. Of interest is the fact that the 18-year-olds average about the same size (10 inches) as the 3- and 4-year-old Cloverleaf Lake fish. The extremely quiescent behavior reported from the Bunny Lake fish suggests a long existence in a state of relatively suspended animation.

Lawrence M. Ashley

GENERAL

Research scientists from other fields studied at Cook to understand basic nutrition, biochemistry, physiology, and metabolism. Dr. Takeshi Nose left in March to return to Japan with a briefcase full of data on 3 projects completed in our laboratory. Papers summarizing data from fish in fresh water and sea water environments were received from Dr. Philip Snodgrass of Peter Bent Brigham Hospital in Boston. Physiology papers from Drs. Gorbman and Oshima from the University of Washington and Tokyo University were edited and submitted for publication. More data on the role of aflatoxin intermediates on cholesterol feedback control were sent to Dr. Siperstein at Texas Medical Center at Dallas. Drs. Maynard Steinberg and Harry Dupree of the Bureau of Commercial Fisheries and the Marion, Alabama Laboratory completed a fat source for a catfish series of experiments which will be reported in detail by Dr. Dupree. Many smaller projects using fish tissue from our experiments were completed by cooperating scientists, results of which will appear in future manuscripts.

A cooperative program to train 6 Lummi Indian candidates in principles and practices of fish husbandry was initiated in October. At the end of calendar 1969, the trainees and instructor were transferred to the Bowman Bay Field Station site to complete the first phase of classroom and laboratory instruction.

FISH GENETICS LABORATORY

Beulah, Wyoming
Bruno von Limbach, Director

HIGHLIGHTS

We have relinquished our limited attempt to breed trout for tolerance of crowding, to avoid further sacrifice of effort on other projects.

Analysis of limited data suggests that both growth and formalin tolerance are strongly heritable and are responding to our selection procedures.

Random matings again show the inherent diversity of available stocks and the potential for selective breeding. For example, similarly reared siblots ranged in average weight from 14 to 333 grams, a 24-fold difference, one year after fertilization.

Performance of a few siblots of 1969 first generation inbred trout (progeny of full sib matings) can be compared with that of their crossbred half sibs. The inbred lots were generally of lower fertility and weighed less at 150 days. Meager data suggest inbreeding depression comparable to that reported for other animals.

Observed phenotype frequencies among progeny of parents of presumed genotype establish the postulated simple autosomal recessive character of albinism in rainbow trout.

Inflow to our four-foot-diameter circular tanks, other conditions remaining standardized, can be reduced from 8 to 4 liters per minute without inhibition of trout growth.

BREEDING RAINBOW TROUT

At the end of the year we have approximately 500 experimental lots totaling 120,000 fish. Roughly 200 lots, or 15,000 fish, are more than 1 year old. Some 3,200 of the older fish bear identification tags to permit individual value assessments and records of performance.

Breeding for albinism

Recent test crosses complete out matings to show that albinism in rainbow trout is a simple autosomal recessive trait. We performed factorial matings, including crosses, incrosses, intercrosses, and backcrosses of the following phenotypes and presumed genotypes: normal color (AA), normal color (Aa) and albino (aa). Observed and expected frequencies of progeny phenotypes are summarized in Table 1 and maximum siblot deviations are footnoted. We also made 24 single-pair matings, 18 intercrosses (Aa x Aa) and 6 backgrosses (Aa x aa). Phenotypically, progeny of these intercrosses were 3,948 (24.7%) albinos and 12,022 (75.3%) normally colored fish; the backcrosses produced 2,402 (49.8%) albinos and 2,417 (50.2%) normals

Breeding for genetic diversity

Breeding to preserve genetic diversity continues. Seventeen random-bred pools of 1965, 1966, 1967, 1968, and 1969 fiscal-year-class stocks are being maintained to produce future generations of unselected brood stocks. Random mating of individuals from 1968 and older year classes for production of 1970 stocks was started in September.

Table 1.--Albinism in rainbow trout:
Progeny phenotypes from 1970 factorial test matings

Number	Matings: Phenotype (presumed genotypes)	Observed Number: Normal	Observed Number: Albino	Observed Percent: Normal	Observed Percent: Albino	Deviation from expected, percent
4	Normal (AA) x Normal (AA)	1415	0	100	0	0
7	Albino (aa) x Albino (aa)	0	6034	0	100	0
7	Normal ♂ (AA) x Albino ♀ (aa)	3259	0	100	0	0
4	Albino ♂ (aa) x Normal ♀ (AA)	1434	0	100	0	0
7	Normal (Aa) x Normal (Aa)	4834	1731	73.6	26.4	1.4 1/
7	Normal ♂ (Aa) x Albino ♀ (aa)	1837	1924	48.8	51.2	1.2 2/
7	Albino ♂ (aa) x Normal ♀ (Aa)	3253	3145	50.8	49.2	0.8 3/
4	Normal ♂ (Aa) x Normal ♀ (AA)	1506	0	100	0	0
6	Normal ♂ (AA) x Normal ♀ (Aa)	5603	0	100	0	0

1/ Greatest deviation from expected ratios produced by a single intercross-Albino 27.9% (258): normal 72.1% (668)

2/ Greatest deviation from expected ratios produced by a single backcross to albino female--Albino 54.8% (86): normal 45.2% (71)

3/ Greatest deviation from expected ratios produced by a single backcross to albino male--Albino 56.3% (291): normal 43.7% (226)

Breeding for variant inbred lines

We are rearing 8 siblots from 1967 and 1968 fiscal-year matings of unrelated parents and 11 siblots from 1969 full sib matings (0.25 inbreeding co-efficient) solely for use in future inbreeding designs, either to start new lines or to further inbreed existing lines. Also, we are utilizing or will utilize a few of the 140 siblots from 1967, 1968, and 1969 matings, made primarily for other purposes, to initiate development of new inbred lines. Breeding of 1967 and 1968 sib lots to produce 1970 lots is underway and we expect to obtain several lots with an inbreeding co-efficient of 0.25 and possibly a few 0.375 inbred lots if certain 1969 year-class males precociously mature at an appropriate time.

In a few instances we can compare the 150-day growth performances of 1969 0.25 inbreds and their crossbred half sibs. An inadequate number of comparisons prevents us from making a quantitative estimate of inbreeding growth depression, but the data suggest that it does occur. We also have some evidence suggesting that we can expect a reduction in fertility as inbreeding progresses.

Breeding for season of sexual maturity

We are rearing 1968 and 1969 year-class lots for selection and breeding to fix or extend differences in seasonal maturity. Lots of both year classes represent parental maturity for each month from September through March. The 1969 lots also include one which was produced by parents that matured in August. Several matings made for April maturity were not successful. Breeding for the 1970 lots was started in August. We have obtained lots for each month from August through December and hope to continue successful breeding beyond March, 1970.

Breeding for age of sexual maturity

Eighty 1967, 1968, and 1969 lots from known-age parents are being reared or maintained for selection and breeding to develop strains or lines differing in age of maturity. Production of 1970 lots began in September. Generally, like-maturing fish, either two-year or three-year maturing, have been mated. However, in a few instances this year we paired one-year-maturing males with two-year-maturing females and three-year-maturing males with four-year-maturing females in initial efforts to develop earlier- and later-maturing lines. We still have only suggestive evidence that female maturity is correlated with the maternal phenotype.

Breeding for growth

Growth was measured for 211 siblots from 1969 matings reared under standard conditions for 150 days after fertilization. The 18 heaviest lots and the 12 lightest were selected for further evaluation. The mean of the 150-day average-fish weights for the heaviest lots, 4.2 grams, was 4.7 times greater than that, 0.9 grams, for the lightest lots.

We have previously noted that wide differences in the growth of siblots, under standardized conditions, from randomly mated as well as from selected parents, have been observed and are indicative of potential for selective breeding. Table 2 shows the average fish weight of the 5 extreme lots measured for each year of our growth selection program. The F. Y. 1967 spawning season data are not directly comparable because we got a late start that year and the matings exclude certain good growing stocks. Other differences in the size of the lots are probably just chance but we can't say that they may not reflect some minor influence of such environmental changes as modified diets and handling methods.

Figure 1 illustrates the difference in size of average fish from the poorest and best 1969 growth lots. They are shown as they appeared after 335 days when their average weights were 10 and 235 grams; a year after fertilization the same siblots averaged 14 and 333 grams.

Available data are insufficient for reliable quantitative estimates of growth heritability or selection response. Most of the 1969 siblots were from matings of unselected parents, as in previous years. Some lots were from parents selected on the basis of their attained adult size. Unfortunately, these spawners had been

Table 2.--The 5 extreme growth siblots of 3 fiscal-year classes

	Siblot average fish weight in grams		
	1967	1968	1969
150 days after fertilization:			
Five lightest	0.53	0.59	0.61
	0.64	0.70	0.73
	0.68	0.78	0.75
	0.68	0.79	0.75
	0.73	0.80	0.83
Five heaviest	2.8	3.2	5.1
	2.9	3.3	5.3
	3.0	3.5	5.3
	3.0	3.5	5.8
	3.6	3.9	6.3
1 year after fertilization:			
Five lightest	25	18	14
	28	18	28
	30	20	29
	30	24	30
	32	32	34
Five heaviest	107	226	232
	114	235	234
	115	240	252
	126	257	253
	176	263	333

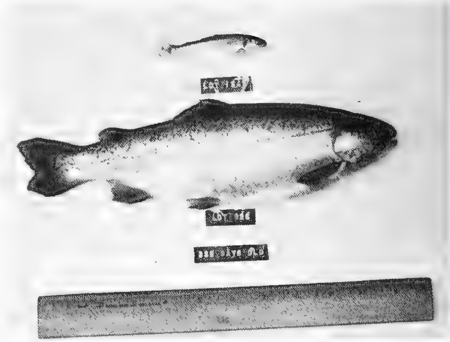


Figure 1.--Average fish from the poorest and best growing sib lots at 335 days of age, weighing 10 and 235 grams respectively.

variously held in dissimilar environments which presumably had a substantial though undetermined influence on their growth.

Among the 1969 siblots there are 15 whose parents both came from 1967 siblots selected on the basis of their 150-day growth. Of the 15 siblots, 6 were progeny of parents from lots selected for good growth and the mean average-fish weight of these 6 progeny siblots is 2.2 grams at 150 days. Similarly, 9 siblots were offspring of parents from lots selected for poor growth and their mean average-fish weight is 1.1 grams. As would be expected if growth is heritable and responsive to selection, these two groups are, respectively, above and below the mean average-fish weight of all 1967 lots, 1.5 grams, not only in terms of group mean average but also with respect to their individual siblot averages. That is, each siblot produced by parents from 1967 high-growth lots has an average weight above the mean average of all 1967 lots, and each siblot generated from the selected poor-growth lots averaged less in weight than the mean of those in the performance base.

In addition there are, among the 1969 lots, 14 siblots whose parents were chosen on the basis of progeny performance, the 150-day average weight of their 1968 offspring which were of course actually sibs or, more commonly, half sibs of the 1969 lots. Preliminary analysis of these data again indicate a similar response to selection for weight, either high or low.

We are breeding 1970 siblots and expect to produce more lots from sib and progeny tested parents. Hopefully they will permit reliable quantitative estimation of the heritability of growth which we suspect is relatively high.

Breeding for crowding tolerance

Matings of 1966 and 1967 fiscal-year-class fish selected on the basis of their growth under crowded rearing conditions for extended periods produced several 1968 and 1969 sib lots. Regrettably, most of these progeny lots could not be tested under crowded conditions. However their growth was measured for 150 days under standard conditions and their performance was not unusual. Two of the 1968 lots were evaluated in a crowded rearing situation for about 4 months; their growth did not differ from that of unselected lots under the same conditions. We have recently abandoned this project because of lack of facilities and manpower for its effective continuation.

Breeding for formalin tolerance

As previously reported, siblot tolerance of formalin is routinely determined from the performance of sample lots exposed for 6 hours to high (525 microliters per liter) and to low (175 microliters per liter) concentrations. Such determinations made for 179 siblots of the 1969 fiscal-year class at about 150 days after fertilization are shown in Table 3, broken down by the several classes of parental selection involved.

From these 179 lots, 18 were selected for retention and future program use: 12 lots, averaging 74% survival at the high concentration appear to be inherently resistant; and 6 siblots, averaging only 32% survival at the low exposure level appear relatively susceptible.

As shown in Table 3, most of the 1969 siblots, 144, were produced by unselected parents but 35 siblots were each the offspring of one (9 siblots) or two (28 siblots) parents that had been selected on the basis of their own (9 siblots), their sibs (14 siblots--8 up and 6 down), or their progeny's (3 siblots) performance. The average performance of the 1969 progeny for every class of parental selection varied appropriately and, except for the one-parent-only lots, substantially from the averages of either all lots or the offspring of unselected parents. Also, it is most appropriate to compare the performances shown for lots produced by parents selected on the basis of their sibs performance with the performance averages of the base population from which the parents were selected--in this case the averages of all tested siblots of the 1967 fiscal-year class. These base population averages are 13% survival of the high test concentration and 80% survival of the low.

Table 3.--Formalin tolerance of 1969 fiscal-year-class siblots

Number of siblots tested	Parental selection	% of sample surviving exposure to test concentration of 525 µl/1		175 µl/1	
		Mean	Range	Mean	Range
144	None (both unselected)	11	0-92	87	16-100
9	None x high sib survival	23	0-96	88	55-100
8	High sib survival	42	0-67	99	96-100
9	Own survival (test age 1½ years)	39	8-87	96	65-100
6	Low sib survival	7	0-20	47	30-50
3	Low progeny survival	10	0-24	51	45-53
179	All lots combined	15	0-96	86	16-100

These comparisons indicate that the tolerance of formalin is a strongly heritable trait. The methods of selection tried appear to be about equally effective at this early stage of the project. As expected, lots with only one selected parent ranged widely in performance and tended toward mediocrity.

If possible, we shall intensify evaluations for formalin tolerance during 1970 by the addition of test concentrations to permit estimation of LC_{50}.

Breeding for DDT tolerance

Sample lots of 142 siblots of 1969 fiscal-year-class fish were tested for tolerance at 150 days post fertilization by exposure for 12 hours to high (40 micrograms per liter) and low (13.3 micrograms per liter) concentrations of DDT in flowing water. Survivors are counted after 24 hours. In general, results indicate some response to selection but it is disappointingly small. Either this factor is not strongly heritable or our selection methods are poor.

For all lots tested, survivals at high and low concentrations, respectively, are 3% and 91%. Included are 97 siblots from unselected parents; these have average survivals of 1% and 93%; no siblot was as resistant or as susceptible as the better performing lots among the 191 unselected 1967 fiscal-year-class lots. The average performance of 11 siblots that were the progeny of one unselected and one selected parent, showed no selection response. Also among the 1969 siblots were 34 siblots from parents selected as follows: 7 siblots from parents surviving exposure as yearlings; 7 siblots from parents selected for high survival of their fingerling sibs; 3 siblots from parents selected for high survival of their progeny; 3 siblots from parents selected for low survival of progeny; and 14 siblots from parents selected for low survival of their fingerling sibs. Combining data for these select lots: the 17 lots from parents chosen for resistance average survivals of 12% and 98% at the high and low concentrations; the 17 lots from parents chosen for susceptibility average 2% and 78%. These percentages, compared with one another or with the survivals of all lots or the unselected lots indicate some response to selection.

METHODOLOGY

Rearing capacities of circular tanks

The feasibility of reducing the rate of inflow to our 4'-diameter circular tanks was investigated. Test situations and results are summarized in Table 4. Loading density levels were periodically restored by cropping. In no instance did a tested inflow rate have any significant effect upon fish growth. The apparent slight depression of growth at the loading density of 35 grams per liter, 40% above our standard, has been noted in some previous studies.

In consideration of these test results, we are adopting 4 liters per minute as our provisional standard inflow rate for 4-foot circulars. This replaces the 8 lpm standard established last year, but we have no basis for modifying other provisional standards.

Table 4.--Growth of rainbow trout in 4-foot circular tanks

Average fish starting weight, loading density, and test duration	Average fish terminal weight, grams					
	4 lpm inflow		6 lpm inflow		8 lpm inflow	
	tank 1	tank 2	tank 3	tank 4	tank 5	tank 6
6 g., 25 g./l., 28 weeks	172	175	---	---	171	177
39 g., 25 g./l., 20 weeks	258	270	260	274	251	294
39 g., 35 g./l., 20 weeks	224	252	234	260	238	255

Exploratory investigations

Fish identification. Many of our 3,200 individually identified older fish are wearing a modified Swedish dangler having a stainless steel tag embossed with 7 digits--4 digits for lot and 3 digits for individual numbers. This year we tried a color code, composed of colored glass "seed" beads threaded on the Carlin-type harness wire, in substitution for the 4-digit lot number. A much smaller stainless steel tag bears only the lot individual number (Figure 2). All of 111 such "tags" applied 8 months ago have been retained and are quite easily read in the course of routine operations such as weighing.

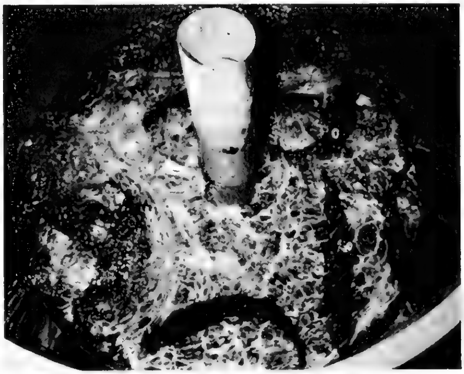


Figure 3.--The use of a numbered plastic disc, seen on fish to the right of screen, attached to the permanent identification tag reduces sorting time and handling during the spawning season.

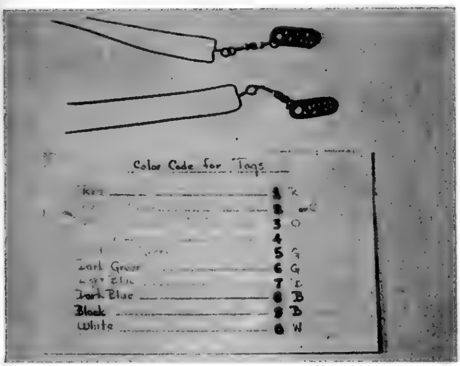


Figure 2.--Colored glass seed beads code the siblot and a stainless steel tag numbers the individual in this new tag for identification of experimental fish.

We are using another innovation to minimize handling during the spawning period. A large colored plastic disc bearing an easily-seen number (Figure 3) is temporarily attached to the permanent tag during the initial screening for ripeness. This permits subsequent recognition of the individual without the necessity of handling it or others in the lot. The "poker chip" is removed when the fish is spawned.

Benzocaine as an anesthetic. Preliminary investigation and limited practical use suggest that benzocaine (ethyl-p-aminobenzoate) is an effective anesthetic for sorting, fin clipping, tagging, and spawning rainbow trout. It can be obtained for about 15% of the cost of MS-222 and our experience indicates that it's anesthetic properties are satisfactory at about half the concentration recommended for MS-222.

Povidone-iodine as an egg disinfectant. McFadden in an article appearing in the Journal of the Fisheries Research Board of Canada (Vol. 26, No. 9, 1969) reported povidone-iodine (PVP-I) to be an effective and safe bactericidal disinfectant for rainbow trout eggs, whereas merthiolate or acriflavin did not destroy Aeromonas liquefaciens on the egg shell. We recently obtained a PVP-I solution from GSA. Inquiry has failed to produce any information on its content or strength. Analysis revealed ca. 1% free iodine, suggesting that it is a 10% solution of PVP-I. Preliminary trials of the GSA product, assuming a 10% PVP-I concentration, at the recommended treatment level (1%) and time (10 minutes) at 12 C produced approximately 50% mortality in eggs treated 15 minutes or 2 hours after fertilization; there was little, if any, adverse effect on eggs treated 17 days after fertilization. At an assumed PVP-I concentration of 2%, eggs treated during the early phases of water hardening were affected less than those treated later; 23% survived the treatment at 15 minutes after fertilization but none survived the treatment at 2 hours or 17 days. We are presently trying to acquire a relatively pure PVP-I solution of known strength for egg toxicity tests. However, we are hoping that McFadden's article will stimulate search by qualified laboratories for a needed safe, effective, bactericidal disinfectant for trout eggs.

Blood measurements for fish characterization. Exploratory investigations of quantitative measurements of blood sugars, bound urea nitrogen, sodium-potassium ratios, and lactate dehydrogenase activity indicate little promise of potential usefulness for practical characterization of rainbow trout stock or lines. Preliminary studies of erythrocyte osmotic fragility and electrophoretic patterns of LDH isozymes were recently initiated.

GENERAL

Construction and maintenance

The shell of the laboratory section of R&D#2, the first small step towards the long-planned major development of this station, was completed well behind schedule and with the usual difficulties and deficiencies. Storage cabinets, shelving, work benches, and miscellaneous furnishings available from GSA have been purchased and set up. Laboratory furniture--base cabinets (but no bench tops), wall cases, and hoods--are almost all delivered but no provision is made for their installation.

Our more pressing need is for fish rearing space. To ease this shortage, an inexpensive pole-type shed, 30' x 72', was erected adjacent to the new R&D#2 laboratory (Figure 4). A 4" AC water main branching from the R&D#2 supply line has been run to the shed. Plans have been made for provision of electrical services, drain system, water distribution system, and addition of two new pumps and controls to the spring-pond pumping plant. When these are provided we plan to install additional stock tanks in the shed and to move 8' tanks from the barn area and from R&D#1 to new locations near R&D#2.

Figure 4. New shed of pole-type construction will shelter fish rearing tanks.

This development will give us a little more space to hold larger fish, facilitate their care by partial consolidation of rearing and spawning units, and afford a little protection against the elements for our staff. In addition it will free some space in R&D#1, where space is needed for fingerling rearing and to install the two stamina tunnels acquired this year. Hopefully they will provide a means of characterizing, perhaps even evaluating our lines and strains of trout.

Cooperative activities

Lectures about our program and tours of our facilities again were given to several hundred family groups who visited us, particularly during tourist season. Sometimes the unexpected visitors who drop in for our regularly scheduled tours include visiting scientists, such as Dr. Erickson, a biochemist from Sweden, and these we enjoy especially. In addition many larger groups were given special tours: the Wyoming Game and Fish Commissioners were here for a somewhat hurried visit, accompanied by some of the staff; our largest group, an estimated 70 persons, were regional members of the American Society of Range Management; youth organizations such as 4-H Clubs, Boy Scouts, and the oddly named Belle Fourche Birthday Boys came to see us; and the usual school groups such as the Sundance High School sophmore class, the senior girls from St. Martin's Convent School, and a group of fisheries students from Athens, Georgia, came with Dr. Fox, the Fishery Unit Leader. The Upper Missouri River Chapter of AFS held business sessions here and toured our station. Of course we also welcome visits from Bureau coworkers, personnel from Fish Hatcheries, Wildlife Refuges, the Minneapolis Regional Office, and the rarer representatives from the remote Bureau Offices in Albuquerque and Washington.

Members of our staff were guest speakers at local service clubs, the Spearfish Fisheries Center, and the Black Hills State College Conservation Workshop. We participated in one session of the Great Plains Fishery Workers meeting in Rapid City.

As usual, fish that became surplus to our research needs were made available to the McNenny National Fish Hatchery for use in cooperative stocking programs. Often we released these lots or individuals with considerable reluctance; our facilities just don't allow us to keep as many fish as long as we would like. In 1969 we transferred 1,250 kilograms of fingerlings (45,000) and 6,950 kilograms of catchable rainbow trout (15,000) to the local management activities.

SALMON-CULTURAL LABORATORY

Longview, Washington
Roger E. Burrows, Director

HIGHLIGHTS

Rolled or pressed dry pellets prepared from the Abernathy diet have proved equal to or better than the soft pellet for salmon rearing.

A starter granule prepared from pressed pellets is superior to the starter mash for first feeding fingerlings.

In release and recovery experiments, significant numbers of the smaller fish have disappeared from the recovery samples, indicating the smaller fish do not survive the downstream migration.

Large fall chinook eggs, less than 99 per displacement ounce, produce fingerlings which incur abnormally high losses due to faulty yolk sac absorption.

Adult returns of fall chinook salmon are 10 to 1 in favor of large fingerlings at time of release.

Unwaterhardened fall chinook eggs survived at approximately the same rate as eyed eggs in this year's incubation channel experiment.

Stocking rates as high as 1,333 eggs per square foot of gravel are under test in the channel.

Coho salmon smolts averaging 17 per pound were released in May after 6 months of rearing in the environmental control system.

Steelhead reared in the environment control system at the Dworshak National Fish Hatchery will easily reach migrant size after one year of rearing while those reared in single-pass river water will require two years of rearing before release.

Comparative commercial pellet prices indicate that the production feeding of Abernathy pressed pellets may result in as much as a 70 percent reduction in the food costs for salmon hatcheries.

APPLIED NUTRITION

Feeding trials for the purpose of developing practical yet nutritionally adequate diets for salmon continued at the Salmon-Cultural Laboratory. This year, instead of continuing with the soft, moist pellet we had developed, we shifted emphasis to the exploration of a completely dry diet which would be suitable for use in salmon propagation. The dry diet, if successful, would be of advantage to salmon hatcheries in that it would eliminate the problems of frozen storage of moist feeds, as well as the difficulties encountered when trying to adapt moist feeds to automatic feeding systems. Dry feeds also should be more economical both in manufacturing costs and in conversion rates.

Two methods of producing the Abernathy dry diet were tested. The first method employed a pelleting process developed by the Food Science Department of the University of Washington in cooperation with the Washington

Department of Fisheries, and consisted of spraying a water mist on the meal and oil mixture of the Abernathy diet while it was being agitated by a rotating disc pelletizer. The spray mist accumulated the meal-oil mixture into a round, rolled pellet, which was then oven-dried and graded to size.

The second method of dry pellet preparation tested was that of pressure or compaction pelleting, a method long in use for the pelleting of dry feeds for trout. One advantage it has over the rolled pellet method is that no oven drying is necessary since no water is added during pelleting. A second advantage is that pressure pelleting is already an established commercial method with a good number of companies capable of making this type of product. With more bidders available, the bidding should be more competitive.

Starting diets

The starter ration we have used in the past and the one we have recommended for use in production has consisted of a mixture of meals, oil, and vitamins. The meals were sized by running them through a 3/32-inch hammer mill screen and then mixing them with the vitamins and oil. This resulting mash performed well as a starting diet for first feeding fish, but has at times been lumpy due to improper mixing on the part of the manufacturer.

Pressure pelleting of the Abernathy diet and then crushing the pellet and screening the crushed particles has made it possible to obtain a small granule that can be used as a starter diet. The small granules can be sprayed or side dressed with additional oil after screening. The result is a starter feed which has a formulation similar to our old starter mash but has the advantage of being an actual granule composed of all of the diet ingredients rather than a loose composite.

A diet trial comparing the old starter mash with the new starter granule was run for 4 weeks using first-feeding fall chinooks reared in constant 53° F. water. The results indicated that the starter granule was a superior diet. The granule-fed fish had a total gain of 146 percent while the mash-fed fish gained 135 percent. All indications are that the starter granule is the best starting diet we have developed so far.

Feeding trials

Our regular feeding trials tested the rolled pellet with the regular Abernathy soft pellet and to determine optimum pellet drying temperatures. Other variables tested included the substitution of other ingredients for cottonseed meal in the basal mix and the use of soybean oil "foots" as a caloric source. The experiment was conducted for 16 weeks and the data indicated the following:

1. The dried rolled pellet was equal to the soft pellet of identical formulation when fed on an isoprotein basis to fall chinook salmon. No problems were encountered with fish accepting the dry pellet, but a smaller sized particle for a given size of fish was necessary.

2. Pellet drying temperatures above 220° F. were detrimental, resulting in a reduction in fish growth. The condition of the fish indicated that the reduced growth was due to an impairment of protein quality rather than a destruction in vitamins.

3. Cottonseed meal could be eliminated from the basal mix and replaced with either corn meal, ground barley, hominy meal, rice bran, rice flour, soybean flour, or wheat middlings, as long as the protein content of the diet was maintained by varying the amount of fish meal.

4. The feeding of soybean oil "foots" as a caloric source resulted in less protein deposition than a similar diet with regular soybean oil. The percent lipid of the flesh of fish fed the "foots" was the lowest of any analyzed.

Although the rolled-pellet method of pellet preparation produces a satisfactory product which is readily accepted by the fish, it does have the disadvantage of requiring added water to pelletize and then removal of water by drying for sizing and storage. It is our opinion that the drying step would add considerably to the cost of pelleting. This added expense would eliminate any cost advantage of a dry pellet over a moist pellet and, since pellet drying temperatures are critical, an exacting quality control would have to be maintained.

To determine if this process was necessary feeding trials comparing the rolled and pressed dry pellets were conducted. After 12 weeks of feeding all indications were that the pressure type pellet was as good if not better than the rolled pellet when fed to fall chinook salmon. In addition, our vitamin analysis showed that the pressed pellet retained a higher level of vitamins after manufacturing than did a similar rolled pellet. The current formula for the Abernathy pellet made by the pressure process and the one recommended for production tests this coming year is shown in Table 1. Granule and pellet sizes are presented in Table 2.

Laurie G. Fowler

Table 1.--Approximate formula of Abernathy dry pellets

Ingredient	Percent	Type
Fish Carcass Meal 1/	41.0	Salmon, dogfish, hake, herring or turbot
Dried Whey Product	23.9	Not less than 15% protein (Foremost or equal)
Cottonseed Meal	15.3	Not less than 50% protein
Wheat Germ Meal	12.8	Not less than 25% protein and 8% lipid
Vitamin Mix 2/	1.0	
Soybean Oil	6.0	Technical grade with .01% BHA and .01% BHT added

1/ Fish carcass meal to have protein content not less than 70 percent, lipid less than 12 percent, water less than 7 percent, and a TBA value below 40.

2/ Vitamin mix as follows:

Ingredient	Amount (grams)
Thiamin mononitrate	0.15
Riboflavin	0.69
Pyridoxine hydrochloride	0.30
Niacin	4.77
d Pantothenic acid	0.68
Inositol	13.65
Biotin	0.03
Folic acid	0.10
DL Alpha tocopherol acetate (10,500 IU)	10.50
Ascorbic acid	25.50
Carrier 3/	397.23

3/ Carrier may be wheat middlings or cottonseed meal sized to pass through a U. S. Sieve No. 20.

Table 2.--Granule and pellet size specifications

Feed Size	U. S. Sieve Size Pass through	U. S. Sieve Size Pass over
Starter granule 1/	30	40
2/64" granule 1/	20	30
3/64" granule	16	20
4/64" granule	12	16
6/64" granule	7	12
8/64" pellet	5	7
12/64" pellet	3-1/2	5

1/ Starter granules to consist of screened fines from 6/64-inch crushed pellets and are to be side dressed with 5 percent soybean oil prior to sacking. The 2/64-inch granule is to be side dressed with 2 percent soybean oil prior to sacking.

EVALUATION OF ENVIRONMENTAL FACTORS LIMITING PRODUCTION IN REARING PONDS

Determination of optimum environments for rearing

Five short-term experiments to determine the effects of different rearing temperatures on growth, body conformation, and physiological characteristics of chinook salmon fingerlings have been completed. In these experiments, groups of fingerlings ranging from 1.38 to 8.94 grams average weight were reared at 50, 55, 60, or 65° F. for 4 weeks. Within this size range, 60° F. appeared closest to the optimum temperature for growth. Temperature apparently had no effect on body conformation or condition factor, and for fingerlings from 3.05 to 17.84 grams, a linear relationship was found between cube of the fork length in millimeters and average weight in grams. Temperature effects on hematological characteristics were observed only in fish averaging four grams or less; increasing corpuscular counts, hematocrits, and hemoglobin levels were associated with increased rearing temperatures.

An experiment is currently underway in which periodic increases in weight and length are being measured at four temperatures, 45, 50, 55, and 60° F. Preliminary data indicate that linear relationships may exist between length increases and time at all four temperatures. If an acceptable and convenient method of predicting growth rates at different temperatures can be developed, close correlation of hatchery feeding levels to these predictable rates should result in improved hatchery production efficiency.

Joe L. Banks

Algacides for use in water reclamation and reuse systems

An algacide is needed for use in water reclamation and reuse systems that will not harm the nitrifying bacterial culture in the filter beds. Four possible algacides, Karmex, Amine D Acetate, Simazine, and GS-13529 were tested in model reconditioning systems during the summer. The models systems were set up outside to encourage growth of filamentous green algae. Karmex was the only product tested which showed any control of the algae at concentrations tolerated by fingerling chinook salmon.

Weekly treatments with Karmex at 4 ppm inhibited the growth of Cladophora but did not eliminate the algae from the system. This concentration is several times higher than that reported to be effective against algae but it is probable that water temperature is a factor. Water temperature in the system never exceeded 70° F. which may be lower than optimum for best results with Karmex or the other algacides tested. The results with Karmex appeared promising enough, however, that we intend to test it under production conditions during the 1970 rearing season.

Bobby D. Combs

MEASUREMENT OF DIFFERENCES IN CHARACTERISTICS OF FINGERLING SALMON

Release and recovery evaluation

This research, begun in 1968, is designed to determine if measurable changes in fingerling characteristics occur within a few days after release and to try to correlate such changes with adult survival. As in 1968, groups of fall chinook fingerlings were cold-branded for identification at several State and Federal Columbia River hatcheries. Samples of these fish were subjected to physical, physiological, pathological, and chemical tests before release. The same tests were performed on marked fish captured on their downstream migration by the Estuarine Investigations of the Bureau of Commercial Fisheries. The fish were usually recovered within a week after they were released from the hatchery.

While fish from only four hatchery releases were recovered this year, several results appeared noteworthy. The first was that none of the recovery groups appeared as severely stressed as those in 1968, with plasma glucose and lactic acid levels near those of pre-release samples. Better condition of the fish before release may account for this difference. Water temperature in the lower Columbia River was lower in 1969 than in 1968, which also would reduce the stress on migrating fingerlings. Lipid losses were again high during migration, ranging up to 50 percent. One of the most striking results was the apparent poor survival of very small fish. In all groups the recovery fish averaged larger than those of the pre-release sample. In one group which averaged 1.5 grams each at release, the migrants captured one week later averaged 2.7 grams or 80 percent larger, indicating that the smaller fish had disappeared.

Bobby D. Combs

Effect of egg size on fingerling growth

Returns of adult fish to our holding pond have increased to a point where we now have an excess of eggs each year. This desirable situation has made it possible to exercise some degree of selectivity over the eggs retained for rearing. The eggs of the returning adult fall chinooks vary considerably between fish in several characteristics, one being the average egg size. In order to determine if egg size is of any significance, selected females of similar length but with large or small eggs were spawned and the eggs fertilized by the same males. The eggs were kept separate, hatched, and are now being reared under identical conditions. Physical data on the individual females as well as results after 6 weeks of rearing are shown in Table 3.

At present fish hatched from large eggs have a greater average weight than do the fish originating from small eggs. Unless there is a change from their present growth rate, the smaller fish will not equal the weight of the larger fish at time of release. An interesting and unexpected development has been the difference in mortality rates between the two size groups. In every instance fingerling mortalities have been greater in fish originating from large eggs. There appears to be an egg size where these losses become excessive. In this experiment eggs which were larger than 99 per displacement ounce had total mortalities after 6 weeks of rearing ranging from 15 to 35 percent. Fingerling losses were caused primarily from faulty yolk sac absorption. It is apparent that egg and fry mortalities are not necessarily indicative of early fingerling losses

Laurie G. Fowler

Table 3.--Physical characteristics of adult female chinook salmon having large or small eggs. Their progeny average weights and mortality rates are for six weeks of rearing

Adult female	Adult female length (cm.)	Total eggs	Number eggs/ displacement ounce	Percent egg and fry mortality	Avg. wt. per fish at start (g)	Avg. wt. per fish at 6 wks. (g)	Percent fingerling mortality at 6 wks.	Total mortalit (%)
1A[1/]	82	4839	139	5.4	0.32	1.34	0.5	5.9
1B[1/]	87	5036	99	2.4	0.40	1.46	3.6	6.0
2A	87	6650	149	1.4	0.30	1.24	0.5	1.9
2B	89	5398	96	5.7	0.41	1.51	10.2	15.3
3A	83	6900	136	4.4	0.30	1.23	2.0	6.4
3B	88	5192	94	9.6	0.41	1.48	19.6	27.3
4A	88	5959	131	6.1	0.33	1.19	0.8	6.8
4B	84	3936	82	10.2	0.45	1.94	28.3	35.6
5A	81	4903	141	2.0	0.29	1.18	1.0	2.9
5B	85	5026	101	3.3	0.40	1.70	4.8	7.9

1/ A and B females represent pairs fertilized by the same males.

DEFINITION OF FINGERLING CHARACTERISTICS NECESSARY FOR MAXIMUM ADULT SURVIVAL

Environmental control systems are capable of producing much larger fish in the same time span than is possible with the colder water available in most single-pass systems. Larger fish require more rearing facilities. Experiments are underway to compare survivals of small creek-reared fish with fish three times as large reared in the environmental control system. While it is possible to produce fish three times as large as normal in the same time span, it is also possible to produce twice as many fish twice as large as normal in about two thirds the normal rearing time, using the same facilities. If such extra fish, released early when pond capacities are reached, should make a significant contribution to the adult run, their rearing would be justified and the contribution of the hatchery measurably increased. The 1969 adult survival experiment was designed to compare the survivals of fingerling fall chinook released March 18, 1969 at 47 per pound with those released April 30, 1969, averaging 21 per pound.

Both groups were reared together in two, 17 by 75, rectangular-circulating ponds. As the pond capacities were approached the fish in each pond were divided in half. One group of 164,201 fish, weighing 3,494 pounds, were marked 1/2 anal and right maxillary and released on March 18. A second comparable group were marked 1/2 anal and left maxillary and released April 30, at which time they numbered 138,450 and weighed 6,460 pounds. Both lots of fish experienced higher-than-normal mortalities as fingerlings due to coagulated yolk and an apparent inherent organic defect in this stock. At time of release there were no recognizable nutritional deficiencies in either group.

Differences in the rates of adult returns between the two groups will be compared with the rates of return of adults from two previous experiments in which normal sized fingerlings and larger fingerlings were released at the same time.

Two-year-olds and 3-year-olds from the two experiments designed to measure the survivals of normal and large fingerlings returned in 1969. The results were most encouraging.

From the 1966 brood year, 152 3-year-olds returned from the 33-per-pound fingerling release and 14 adults from the 96-per-pound fingerling release--more than 10 to 1 in favor of the larger fingerling at release. While this ratio of return may change slightly when the 4-year-olds are accounted for, it is not anticipated the change will materially alter the very significant difference in favor of the larger fish. A 10-fold increase in the number of returning adults more than compensates for the additional capital outlay and operating costs of an environmental control system. Returns of such magnitude also require an increase in the adult harvest.

The returns of 2-year-olds from the 1967 brood year are even more encouraging. At this laboratory a large jack run has always been indicative of a high survival of this particular year class and has been correlated with increased adult returns. The return of 2-year-olds in 1969 from the fingerlings reared in the environmental control system has been phenomenal.

In this experiment, two groups of 200,000 fingerlings, each, were reared in single-pass creek water and in the environmental control system at water temperatures averaging 56° F. Both groups were marked with a common 1/2 dorsal clip and the removal of part of either the left maxillary for the 26-per-pound fish reared in the environmental control system or right maxillary for the 80-per-pound fingerlings reared in creek water. Releases were made on May 15, 1968.

In September and October of this same year we recovered 104 marked, sexually-mature, male yearlings from the adult holding pond. These fish were all from the 26-per-pound fingerlings reared in the reuse system. At time of capture they weighed about one pound apiece. While mature yearlings are not unusual we had never recovered them in such numbers before.

In 1969, both groups were recovered from the sport fishery off the Washington Coast. A total of 374 jacks were reported with the 1/2 dorsal, left maxillary mark and 3 fish with 1/2 dorsal, right maxillary mark. It is estimated that from 1 to 3 percent of the total chinook sport catch off Westport, Washington was composed of jacks from this single Abernathy release.

The 2-year-old return to the Abernathy holding pond was equally surprising. There were 448 fish, including 7 females with the 1/2 dorsal, left maxillary mark, and 6 fish, all males, with the 1/2 dorsal, right maxillary mark. We have never had a marked 2-year-old return of this magnitude before. In fact, the largest previous return was in 1968 and amounted to 29 fish, also reared in the environmental control system.

Needless to say we anticipate some record-shattering adult returns from this group of fish in 1970 and 1971. Unless some major ocean catastrophe occurs, all the evidence indicates that such will be the case.

ABERNATHY INCUBATION CHANNEL

Effect of egg development at planting on egg and fry survival

The 1968-69 channel experiment involved eggs from 120 chinook salmon females from a single day's take in late September, 1968. Eggs from 40 females were planted in separate experimental areas as (1) unwaterhardened, green eggs, (2) waterhardened, green eggs, and (3) eyed eggs. Fry migration extended from December, 1968 until operations were terminated in mid-March, 1969. Migrant survivals from the channel were: (1) 75.9 percent from the unwaterhardened, green eggs, (2) 60.2 percent from the waterhardened, green eggs, and (3) 77.6 percent from the eyed eggs. Earlier tests with eyed eggs had produced survivals which varied from 68.0 to 78.5 percent, similar to the present results. In the previous season, unwaterhardened, green eggs had a 50.1 percent survival and waterhardened, green eggs had a 37.6 percent survival. The present tests indicate that the planting of unwaterhardened, green eggs in incubation channels shows promise as a practical production procedure.

Effect of various egg planting densities on egg and fry survival

The recommended maximum egg-stocking densities for spawning channels vary from 139 to 167 eggs per square foot of gravel. The Abernathy Incubation Channel has used stocking rates as high as 435 eggs per square foot of gravel with high fry survivals. This stocking rate was based upon the number of eggs planted in sections of the channel and included areas not utilized for eggs, such as drop structures. If based upon the actual area of gravel utilized for eggs--20,000 eggs per 10-foot trench with centers 36 inches apart--the density of eggs would be 667 eggs per square foot of gravel.

Using the 667 egg-density as the control, the 1969-70 experiment seeks to determine the maximum stocking density per available square foot of gravel which will not result in reduced egg and fry survivals. About 600,000 eyed eggs of fall chinook salmon were received from the Spring Creek National Fish Hatchery in early November 1969. The eggs were found to have a fairly high incidence of "soft-shell" disease. To correct for this condition all lots of eggs were thoroughly mixed before planting. In this way, any resulting mortality should occur uniformly throughout the channel and will not be interpreted as a result of planting density. Survivals may be lower than in previous years, but comparisons between eggs planted at various densities should still be valid.

The eggs were divided among the three experimental areas and planted at densities of (1) 667 eggs per square foot using 36-inch centers for trenches, (2) 1,000 eggs per square foot using 24-inch centers, and (3) 1,333 eggs per square foot using 15-inch centers. The 1,333 egg-density appears to be the maximum stocking rate physically possible for the channel. Evaluations will begin in late January, 1970 when the first migrants begin leaving the channel.

Mechanisms of migration

Studies were begun to determine the influence of environmental factors of emergence and downstream movement of salmon fingerlings. Eight wooden troughs were constructed to simulate conditions found in the incubation channel (Figure 1). The arrangement of water supplies and filters was designed to test the effects of water temperature, turbidity, and changes in water flow. The first four troughs were designed to test the effects of artificially-induced increases in water temperature, water flow, and turbidity.

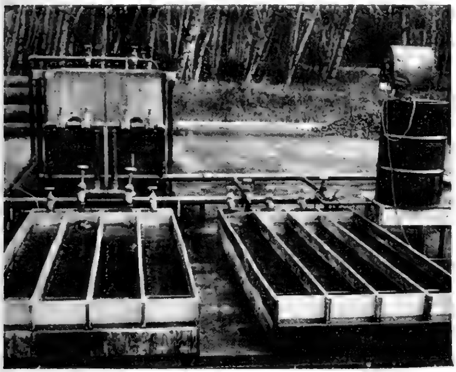


Figure 1.--Simulated incubation channels for the study of triggering mechanisms causing fry migration. Sand filters in background. Barrel at right for introduction of silt. Troughs at left test effects of filtered and unfiltered creek water at two flows. Troughs at right test effects of increases in water temperature, flow, and turbidity.

In late October, 2,000 eyed eggs from Abernathy Creek fall chinook salmon were planted in each trough at stocking rates comparable to the density normally used in the channel. Migration from the channel models is expected to extend from mid-January to late February. The effects of each variable will be evaluated by the pattern of the fry migration.

Allen E. Thomas

EVALUATION OF STRESS IN FINGERLING SALMON

Exercise as a stress factor

Two experiments were conducted to test the effects of water current extremes in the rearing environment on the physical, hematological, and chemical characteristics of fingerling chinook salmon. In the first experiment, groups of fish were reared in a "fast-flow" circular tank and a "slow-flow" trough. Disease in both groups forced abandonment of the tests after 4 weeks. The effects of disease were more prominent in the exercised group, as shown by higher mortalities, reduced growth, lower hematocrits, and reduced swimming ability.

In the second experiment, a fish population was split and placed in two circular tanks, one with a fast water current and the other with essentially no current. Evaluations were made after 6 weeks of rearing. Individual variations and the small sample sizes prohibited statistical analysis in most cases. The exercised group, however, developed higher stamina, blood counts, hemoglobins, liver glycogen, and lactic acid than did the non-exercised group. Exercised fish also were about 6 percent smaller, although the percent body fat and condition factors were the same for both groups. Future tests will involve larger numbers of fish and duplicate tanks for more valid statistical analysis and will more closely investigate the effect of exercise on growth.

Allan E. Thomas

GENERAL

The Sixties have proved to be a most productive and rewarding decade for this laboratory, productive in that the rectangular-circulating pond, the environmental control system, and the Abernathy dry diet were developed and tested, and rewarding in that all of these developments have been applied in hatchery operations. One of the most frustrating experiences for a researcher is to develop something which has practical application and then not have it used. We have been most fortunate in being able to work with men in both our Bureaus and in several of the western states who have been most progressive and willing and able to apply in production operations the results of some of our research and development.

As a result, the rectangular circulating rearing pond is being used in new and remodeled Oregon Fish Commission hatcheries and at several National Fish Hatcheries. Small environmental control systems are being used at the Little White Salmon and Coleman National Fish Hatcheries with the first large and complete installation at the Dworshak National Fish Hatchery. Two other systems are nearing completion, one at the Fire Lake Hatchery of the Alaska Department of Fish and Game and another at the Mad River Hatchery of the California Department of Fish and Game. Several more environmental control systems are in the design stage.

The Abernathy dry diet, pressure pelleted, is being fed on a production test basis to 25 percent of the fall chinook fingerlings being reared at the Columbia River hatcheries of the Bureau of Sport Fisheries and Wildlife. In addition, the Washington Department of Fisheries is conducting extensive tests at six salmon hatcheries to compare the Abernathy diet as either pressed or rolled pellets.

The year 1969, itself, has been especially interesting. The winter was severe with snow on the ground from January through most of March, with relatively cold weather, down to 18° F. for some of this time. No difficulties were experienced from the freezing conditions but the snow accumulation had to be removed from the laboratory roof at one time to safeguard against collapse. The remainder of the year was normal with enough precipitation in September and October to provide an adequate flow in Abernathy Creek for returning adults.

The adult return was excellent, considering that it coincided with the opening of the gill net season on the Columbia River. Despite the large commercial catch of fall chinook salmon, we took over 3,000,000 eggs, retaining approximately 1,500,000 and shipping the remainder as eyed eggs or first-feeding fingerlings to the Grays River hatchery of the Washington Department of Fisheries.

From the previous brood year, 16,000 pounds of fall chinook fingerlings averaging 62 per pound and totaling 640,000 fish were released into Abernathy Creek. In addition, 78,400 coho fingerlings weighing 4,730 pounds and averaging 17 per pound were released the latter part of May. The coho were from 1968 eggs and had been brought to smolts in 6 months of rearing in the warm water of the environmental control system. These fish were marked by feeding tetracycline and removing the adipose fin. Adult returns will indicate whether this is a practical production procedure.

The selection of eggs and fry of fall chinook salmon for production rearing continued during 1969. The eggs from females larger than 80 cm. (31.5 in.) were held separately and culled on the basis of fecundity, viability, and egg size. The range of egg sizes from the selected fish was from 71 to 148 per displacement ounce. The range of size of the selected eggs was from 71 to 130 eggs per ounce. All lots with an egg and fry mortality greater than 10 percent were culled and lots with less than 3,500 eggs per female were discarded.

We are beginning to doubt the wisdom of retaining abnormally large eggs for rearing. As indicated in the egg size experiment, extremely large eggs produce defective fingerlings with a high death rate. The disease appears as a type of coagulated yolk with losses in the early fingerling stage. Symptoms are similar to those of "cold water" disease but where the eggs of individual females are isolated the disease is confined principally to the large egg lots. In production, dead fish will be spotted, with some ponds experiencing much higher mortalities than others, depending on the distribution of the affected fingerlings.

We have assured quality control for the Abernathy diet when used in production in order to assure a uniform product for large-scale tests. Proximate analyses are made of samples of all ingredients prior to manufacture and of the processed granules and pellets. Analyses are made to determine protein, lipid, carbohydrate, ash, and water, as well as selected vitamins. Specifications are so written that any ingredient or the formulated diet may be rejected if the requirements are not met. Usually we have encountered no difficulties but on one occasion the manufacturer had inadvertently omitted the entire vitamin package from the soft pellet. As a result, 30,000 pounds of soft pellets had to be reprocessed but about 1,000,000 fall chinook fingerlings were saved. Quality control is a most necessary adjunct of commercial pellet manufacture.

Small tank feeders as shown in Figure 2 have been purchased to automate the feeding of the fish on experiment. At a cost of $3,200 we have automated the feeding of 70 circular tanks. Such automation makes it possible for one man to handle the diet trials alone and eliminate the necessity for a biologist to be on Saturday and Sunday duty. Timers on control panels make it possible to control both the number of feeds and the amount fed per feeding.

Figure 2.--Tank feeders used for dry feeds.

A working model of the environmental control system, as shown in Figure 3, was designed and assembled at this laboratory. The model was constructed primarily for display purposes at the Boy Scout Jamboree but has since been used at the dedication of the Dworshak National Fish Hatchery and the Chelan County Fair. All reports indicate it to be a very satisfactory exhibit. It is scheduled for other showings in 1970.

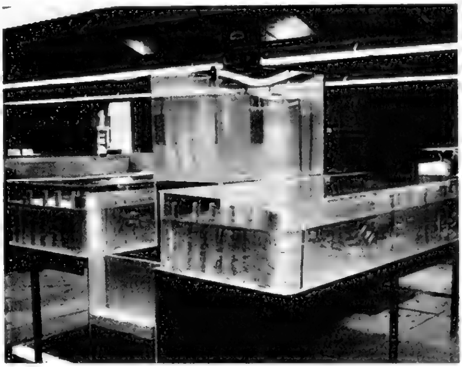


Figure 3.--Working model of environmental control system showing refrigeration unit and aeration chamber above, rectangular-circulating ponds at sides, and oyster shell and rock filter below.

All production environmental control systems are working well. The largest and most complete system now in operation at Dworshak, is proving to be entirely practicable. The 1969 steelhead fingerlings in the system are averaging 17 per pound at present. There is no question but that they can produce downstream migrants by May. Fish reared in the river water, in contrast, will have to be held an additional year before release. No major problems in design or operations have been encountered.

Tests of the Abernathy diet in production have proved most satisfactory. Fall chinook fingerlings at the Quinault N.F.H. were reared exclusively on the Abernathy diet, pressure pelleted. The fish were in excellent condition at time of release. Coho fingerlings, also, are being reared at this station on the Abernathy pressed pellets with no problems. Steelhead at the Dworshak National Fish Hatchery have been reared for seven months to date on this diet with excellent results. The cost of the pressed pellet is 11 cents per pound by commercial processing, in contrast to 16.9 cents for the moist pellet. The dry pressed pellet is only 50 percent the cost of the moist pellet and requires 20 percent less feed to produce the same poundage of fish. Feeding of the Abernathy pressed pellet in salmon hatcheries can result in as much as a 70 percent saving in food costs.

Roger E. Burrows

SIERRA NEVADA AQUATIC RESEARCH LABORATORY

Bishop, California
Norman Reimers, Director

HIGHLIGHTS

Immunological comparisons of bloods suggest a separation between a hatchery broodstock and a selected line developed from it by breeding survivors of stream tests. The third generation of the experimental line also continued to exhibit more wildness than its control, but did not survive a long-term stream exposure as well as the control.

Trout were successfully cannulated for vascular physiological studies using a recently demonstrated technique, after several other procedures had proved unsatisfactory in tests. Chronic maintenance of trout with indwelling arterial and venous cannulae is now an available method.

In a program of tests designed to evaluate the efficacies of different soluble anesthetics, MS-222 significantly affected liver glycogen levels for critical post-anesthesia periods. It is therefore considered unsuitable for use as a stress restraint in tracer studies of carbohydrate metabolism.

Our guinea pig colony, now bred through enough generations to be thoroughly altitude-adapted, produced several valuable immune sera and biologically useful blood fractions for use in the developing "physiology of adaptation" program. Rats and rabbits were added to the antibody factory this year.

Partial results of a long-term experimental steroid stressing program, begun this year, suggest the possibility that measurable, stress-mediated leukopenia may be useful in evaluating adaptational success in fish.

Preliminary experiments dealing with stream feeding suggest that learning plays a role in efficiency of natural food utilization.

We now have evidence that night feeding by hatchery trout in streams accounts for a significant percentage of their daily food intake.

Our stream studies suggest that winter conditions lead to poor feeding by reduction of behavioral drift of insects rather than reduction of feeding efficiency.

Movement experiments indicate that certain aspects of trout-planting technique, such as time of day plants are made, the place and manner of planting, and nutritional state of fish at planting, significantly affect their ultimate location in a stream.

Study of winter feeding habits of brook and rainbow trout in high alpine lakes indicates a high level of activity under heavy ice and snow cover. Loss of the very important midge pupae from the food supply soon after lakes freeze greatly reduces winter feeding success, compared to that in ice-free periods.

We now have all data needed to describe a 19-year life history of a single generation of stunted brook trout. This attained age is four to five times the normal lifespan of the species.

SURVIVAL AND VITALITY OF TROUT

Survival selection of rainbow trout

Long-term stream tests of initially matched groups of catchable-sized trout (Hot Creek fall-spawning broodstock, and fish bred from selected survivors of the same stock through two generations; both stocked as mixtures at 100 lb./acre in two closed natural stream sections) began in July, 1968, and ended in May, 1969. We found no change from the previous generation in the relative performance of the two groups. That is, F_2 experimental fish followed essentially the same seasonal course of survival as broodstock controls in this test as did F_1 experimental fish in the 1965-1966 test. Table 1 indicates this course in terms of mid-term and final censuses. The selected groups appeared to have a slight advantage in the early months, but survived the winter and spring at lower rates than hatchery stock in both years.

Table 1.--Comparative survival of selected (F_1 and F_2) and broodstock (B) types of Hot Creek rainbow trout, after summer stocking at 100 lb./acre

Trout group and Year	Percent survival		
	Aug.-Nov.	Nov.-May	Aug.-May
1965-66			
B	83.4	55.5	46.3
F_1	86.3	44.0	37.7
1968-69			
B	92.8	27.7	25.8
F_2	96.9	17.5	16.6

In both experiments, the "survival-bred" trout were spawned from parents that had survived a stream test in relatively superior condition. Our objective was to demonstrate selection back toward wildness in a highly domesticated hatchery stock, hopefully improving post-hatchery survival in the process.

At this point (3rd generation) we have not found evidence that such selection has survival value in the Hot Creek stock, which supposedly has a very low genetic variability coefficient. However, we have some evidence that a distinguishable change of type is taking place within the stock as a result of the selection (see Physiology Section later in this report). We have also observed what appear to be indications of greater tendency to wildness in the selected type. During a holding and feeding period following recovery from the stream, "survival-bred" trout were definitely more flighty and nervous, less inclined to accept prepared feed, and more susceptible to infestation by pathogenic protozoans. Any methods for verification of selective change will be useful in further studies of type distinction and altered adaptive potential in hatchery trout.

Performance and other tests of two California hatchery trout strains

Stream Sections 1 and 3 were stocked in late September with mixtures of equal weights to total an initial 100 lb./acre of catchable-sized Hot Creek and Mt. Whitney rainbow trout. Additional rainbow trout from the two lots, and wild brown trout of comparable size, were installed in indoor holding facilities for maintenance feeding and later comparisons with stream groups.

Purposes of these exposures are (1) to compare survival in the stream with that of other marked trout from the same lots that were stocked in lakes by the California Department of Fish and Game, and (2) to compare stress responses in biochemical terms among the available situations.

November samples of 10 fish per group were reserved for proximate and other analysis, together with initial, additional mid-term, and final samples. Observed mortality in the stream has been low to the end of the year.

Record-age brook trout of Bunny Lake

The original stocked generation of this small experimental population is now in its 20th (and, from all indications, final) year of life in a high-altitude cirque lake. Some highlights of previous work with these fish, whose age over the past several years has been unprecedented from our knowledge of the species, have been mentioned in earlier annual progress reports.

Eleven of the few remaining specimens were removed by angling and transferred to the laboratory in October. One of these, shown in Figure 1, bore the evidence of its age in the stump of a pelvic fin that had been amputated in 1952, when about 150 of the initial 1,800 trout were so marked.

Figure 1.--Bunny Lake brook trout taken in 1969. Age of 19 years verified by stump of fin (shown between hands) removed in 1952.

All possible general work on the ecology and ordinary histology of these aged and stunted fish has now been completed. Some of this year's findings were:

1. A slightly higher index of abundance was observed for some of the invertebrate food forms that had disappeared following heavy food consumption early in the history of the trout group and that have reappeared in small numbers during the past three years. Neither of the two genera of larger zooplankters (Daphnia and Diaptomus, both rendered extinct by overcropping in 1953) were in evidence, however, and it is now clear that such small, oligotrophic lakes may suffer long-term and possibly permanent faunal alterations following continued overpopulation by trout.

2. Individual fish in the present remnant of the age-group apparently received no new growth advantage due to their now rapidly dwindling number, although increased growth was attributable to marked population reduction and increased feeding at earlier ages, 12 to 15 years. (See Figure 2, which shows two segments of greater growth rate and two periods of retardation during the long life-history. Length data from preserved fish, added to the plot, flatten the 1957-61 portion of the curve to a better approximation of non-growth for this period.)

A laboratory-maintained individual grew only 1.8 cm in length and 1.3-fold in weight from October, 1968 to October, 1969 with regular brine shrimp feeding, suggesting that extremely advanced age by itself may be a final curb on the potential for growth. Another lab-maintenance growth test was continued for 20 months with a 12- to 14-year-old Bunny Lake specimen in 1962-64. The results (11.3 cm length increase and 5-fold weight increase) were much more impressive, but I cannot be certain whether an effect of lesser age or an advantageous individual capacity for growth was in control.

3. A report on the age-indicative condition of representative tissues from age-group XVIII trout was received from Dr. L. M. Ashley, collaborating histopathologist at the Western Fish Nutrition Laboratory, in March of this year. This second analysis was similar to an earlier one made at age 13 in that no really definitive differences could be found between fish of extreme age and those grown at normal rates to age 3 or 4 years.

Materials for confirmation of chronologic age--other than fin-clips providing known-age reference--are on hand but have not yet been analyzed. We plan to make tests of collagen alteration as an indicator of aging and to attempt the interpretation of otoliths.

Future work, utilizing reduced organic principles and other material collected this year, plus whatever may be left at the lake in the coming summer, will be more specialized and will be concerned with indicative histochemistry, enzyme assay, and other biochemical evaluations to improve our knowledge of

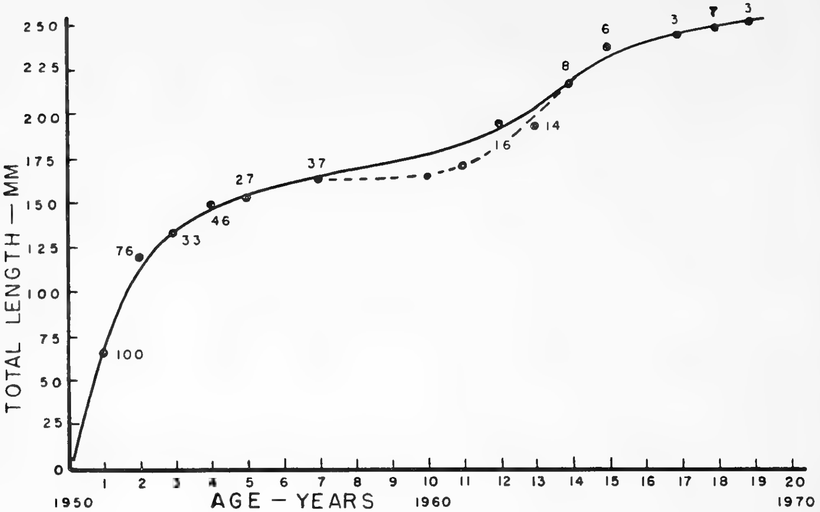


Figure 2.--Lifetime growth of Bunny Lake brook trout as estimated by available sample measurements (sample sizes shown at points). Broken-line section represents addition of 1960 and 1961 length data from preserved fish.

age changes in freshwater salmonids and to explore the possible implications for alpine trout management.

Norman Reimers

PHYSIOLOGY AND BIOCHEMISTRY

Serodiagnosis of F_2 survival bred trout

In order to test the hypothesis that the genetic constitution of the Hot Creek strain of rainbow trout could be environmentally altered to enhance stream survivability, the second generation (F_2) of line-bred stream survivors was subjected to serodiagnosis. The data suggest that some serological (=genetic?) differentiation between the parental hatchery stock and the second filial generation of inbred stream survivors may have occurred.

The blood of five F_2 stream survivors was pooled and fractionated to obtain antigens for immunological challenge of guinea pigs.

Three groups of five guinea pigs each were immunized against these F_2 blood fractions:

(I) Whole plasma proteins

(II) The supernate fraction of osmotically lysed and centrifuged (15,000 x g) erythrocytes (erythrocyte hemolysate).

(III) The finely divided 15,000 x g pellet material.

The respective antisera were titered by agar gel diffusion in the case of fractions I and II, and by erythrocyte agglutination for fraction III. This latter fraction proved antigenically undependable; fraction I was strongly antigenic, but the immunological responses of the five guinea pigs so challenged were too highly individual to be serodiagnostically reliable. Serial dilution of fraction II in agar gel diffusion tests against a pooled antisera, composed of the sera of the three most immunologically competent guinea pigs, selected out the most antigenically significant patterns.

The cross-reactivity of the antisera with blood cell fractions obtained from Hot Creek Hatchery rainbow trout, the Mt. Whitney Hatchery strain, and the brown trout was determined by serial dilution in agar gel. Figures 3 and 4 contrast the antigenic properties of the red blood cell preparations of the four fish-types studied. Figure 5 is an Analytrol tracing of Microzone electropherograms of the whole plasmas of the F_2, Hot Creek and Mt. Whitney rainbow trout. Further tests to confirm the suggested emergence of a variant strain as a result of line breeding of selected stream survivors will be possible when the F_3 generation becomes available in fall, 1971.

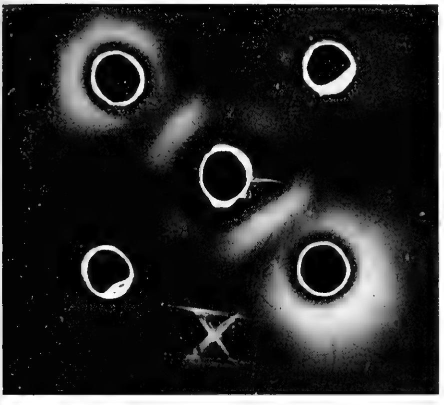


Figure 3.--Agar gel diffusion pattern of immunological responses between antisera to F_2 erythrocyte hemolysate (center well) and the following antigens, diluted to approximate "equivalent combining proportions":

a) F_2 rainbow erythrocyte hemolysate (upper left and lower right)

b) Hot Creek rainbow erythrocyte hemolysate (upper right and lower left)

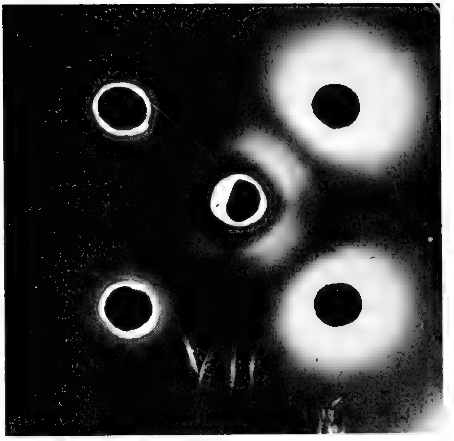


Figure 4.--Agar gel diffusion pattern of immunological responses between antisera to F_2 erythrocyte hemolysate (center well) and the following antigens, diluted to approximate equivalent combining proportions:

a) Brown trout erythrocyte hemolysate (upper left)

b) F_2 rainbow erythrocyte hemolysate (upper right and lower right)

c) Whitney rainbow erythrocyte hemolysate (lower left)

Survey of lakes and streams for environmentally-produced developmental defects in trout

In collaboration with Dr. Bernard Baird, University of California at Berkeley, a teratogenic survey of hatchery fry and fingerlings are being compared with young-of-the-year wild trout obtained at 9,500 ft and 10,500 ft altitude from two representative Sierra Nevada drainages this year. When statistical and histopathologic data are correlated, physiological experiments can be designed to test the extent to which hypoxic stress can affect pre- and post-hatching developmental processes. It is hoped thereby to assess the role of natural recruitment in maintaining a healthy and vigorous population of fishes of various strains and species in marginal habitats.

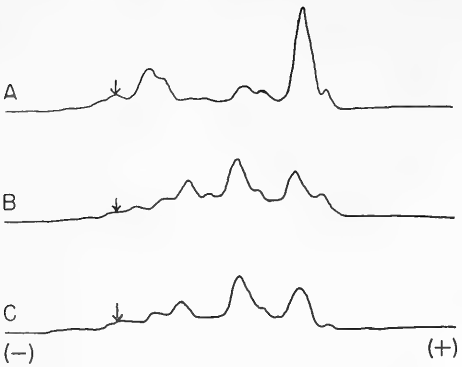


Figure 5.--Analytrol scan of Microzone electropherogram of the plasmas of three test groups of rainbow trout.

A. Whitney strain rainbow
B. F_2 survival-bred rainbow
C. Hot Creek production rainbow

The individual samples (pooled from 5 animals each) were run on the same cellulose acetate membrane. Sample size 0.5 µl; 30 minute run @ 250 v (2.5-4.5 mA) in pH 8.6 barbital buffer, µ=0.05. Arrow indicates origin. Polarity indicated by (+) and (-).

Extraction and isolation of somatotropin from salmonid pituitary glands

Although we were unable to obtain several thousand salmon pituitary glands as planned this year, 1,154 pituitaries collected in the autumn of 1968 were defatted, lyophilized, and divided into several lots of finely powdered material. We have tested microanalytical methods and are able to identify and quantitate aliquots of peptide fractions eluted from chromatographic columns. At present we are working out the most efficient and least destructive method of extraction so that we can obtain other trophic peptides in addition to growth hormone. This can be characterized as a "pilot plant" approach to the extraction, isolation, and partial chemical characterization of pituitary trophic hormones, antecedent to the understanding of the physiological role of the pituitary gland in the adaptation and survival of hatchery-reared trout in montane and alpine waters.

Immunoassay of insulin in the salmonid circulatory system

The survival of hatchery-reared trout released into wild waters requires certain metabolic accommodations. Carbohydrate metabolism can be easily upset by the stresses imposed by the new environment, although the extent of this metabolic upset is conjectural. Blood insulin levels, in association with amounts of circulating catecholamines and glucocorticoids, will give valuable insights into the process of adaptation. To assay for insulin, we shall use a double antibody radioimmunoassay. As the first step, we have obtained high-titer antisera to crystalline bovine insulin from guinea pigs that were carried on an immunization program earlier in the year. (Fig. 6.) Guinea pig anti-insulin, however, is a non-precipitating complex. At present an antibody against guinea pig gamma globulin is being formed in immunized rabbits.

Upon receipt of our A.E.C. radioisotope-use license early in 1970, we shall be in a position to label purified insulin, prepared from crystalline commercial bovine insulin, with ^{125}Iodine and ^{131}Iodine. The facilities, equipment, and supplies to conduct large-scale insulin radioimmunoassays were built up this year and are in readiness.

Effect of steroids on the salmonid hematopoietic system

Avian physiologists have used corticoid-mediated leukopenia as an indicator of stress in birds undergoing a variety of environmental manipulations. If the technique were applicable to fish, the degree to which an animal was successful in adapting to a habitat could be gauged.

In collaboration with Dr. Russell R. Burton, University of California at Davis, we have undertaken a study to determine the following:

Figure 6.--Blood is withdrawn from anesthetized guinea pigs to supply gamma globulin and other serum components.

(1) Do elevated blood levels of steroids induce a significant leukopenia in trout?

(2) Could a leukocyte count be used as a reliable diagnostic indicator of stress or condition?

(3) Is the stress response, if any, temperature-dependent?

Testosterone propionate is used as the challenge because of its low cost-per-dose factor as well as its demonstrated increased ratio, erythrocyte count/leucocyte count, in avian species. Dose-body weight tests have shown that the intraperitoneal administration of 10 mg testosterone propionate per 100 g body weight is well tolerated, even 2 to 3 times per week. (Care must be taken, however, to avoid the use of commercial preparations containing ethyl or benzyl alcohol since we have encountered significant mortality in experimental lots exposed to such preparations.

The experimental design involved the maintenance of 3 groups of 30 rainbow trout each on 3 regimens:

(1) Steroid-injected fish receiving a total of 75-100 mg testosterone propionate over a 1-month period prior to sacrifice;

(2) Control fish injected with volumes of isotonic saline equal to the volume injected into the fish on the steroid regimen;

(3) Uninjected controls.

Upon termination of an experiment all fish are killed and 3 blood smear slides per animal are prepared. Four series of experiments were designed to test the influence of water temperature on the experimental results:

(1) An experiment conducted in September when water temperature ranges between 14 and 17°C.

(2) An experiment in November at 6 to 8°C.

(3) An experiment in February at 0.5 to 3°C.

(4) An experiment in May at 8 to 12°C.

The first two experiments have been completed and are currently being analyzed. Analysis of variance of random samples taken from the September experiment, shown in Table 2, indicates that the leukocyte count is a highly individual characteristic for fish. There is a suggestion of steroid-mediated leukopenia; however, a more definite statement must await further analysis.

An extension of this study is the development of an indirect method of total leukocyte count. The method appears statistically valid on preliminary investigation. If it proves valid, it will be a useful adjunct to our field program since it will eliminate the need for cumbersome equipment.

Table 2.--Leukocyte count of blood smears obtained from randomly selected rainbow trout injected with 75 mg testosterone propionate (STEROID), an equivalent volume of isotonic saline (SALINE), or uninjected (CONTROL) during September, 1969 (water temperature range 14.5-16.3°C.). Analysis of variance: $F_{.99} = 3.46$.

Animal Identification	Slide #	# Leukocytes counted	# Fields examined	Leukocytes per field	Regimen
A	2	65	30	2.17	STEROID
A	1	53	25	2.12	STEROID
A	3	52	25	2.08	STEROID
H	1	119	25	4.76	STEROID
H	3	102	25	4.08	STEROID
H	2	114	25	4.56	STEROID
I	1	190	25	7.60	STEROID
I	2	111	26	4.30	STEROID
I	3	106	25	4.20	STEROID
J	1	54	25	2.20	STEROID
J	2	42	25	1.70	STEROID
J	3	52	25	2.10	STEROID
O	3	116	25	4.64	SALINE
O	2	85	25	3.40	SALINE
O	1	115	25	4.60	SALINE
Q	1	83	25	3.30	SALINE
Q	2	136	25	5.40	SALINE
Q	3	139	25	5.60	SALINE
R	1	101	25	4.00	SALINE
R	2	100	25	4.00	SALINE
R	3	95	25	3.80	SALINE
L	1	339	25	13.60	SALINE
L	2	291	25	11.60	SALINE
L	3	336	25	13.40	SALINE
X	1	250	25	10.00	CONTROL
X	2	250	25	10.00	CONTROL
X	3	267	25	10.70	CONTROL
AA	1	128	25	5.10	CONTROL
AA	2	173	25	6.90	CONTROL
AA	3	122	25	4.90	CONTROL

Effects of anesthetics on carbohydrate metabolism

In order to administer radioactive tracers to fish for *in vivo* studies of carbohydrate metabolism, a method of restraining the animal for injection while avoiding stress-induced alteration in blood and tissue carbohydrate levels must be used. We are currently searching for a chemical agent which will rapidly immobilize a fish, have no significant effect on short term (less than 30 min) carbohydrate metabolism, and work well at low water temperatures (0.5 - 7°C.).

The barbituric acid derivatives, sodium barbital and amobarbital, were unsatisfactory on the basis of prolonged duration of induction as well as the evidence for hepatic involvement in metabolism of these drugs. The action of 2-phenoxyethanol, at 1:4500 and 1:9000, was unpredictable at low water temperatures, causing a violent contact reaction by fish, variable blood glucose responses and incomplete blockade of pain responsiveness. Paraldehyde, 1:3800, was similar in action to 2-phenoxyethanol.

Many workers have found MS-222 the most efficacious anesthetic for fish. Our investigations indicate, however, that this popular agent is undesirable for use in studies of carbohydrate metabolism which require repeated blood sampling of the sedated animal over a short duration following induction. Figure 7 presents the results of one of several experiments in which liver glycogen levels were significantly affected by maintenance of fingerling rainbow trout (av. wt = 30 g) in piped stream water made up to 1:15,000 MS-222, pH 7.0, at 2.8°C. Blood glucose responses, while individually too variable to be significant statistically, are suggestive of endocrine involvement. In progress now are studies of the effect of MS-222 on the circulating levels of epinephrine and cortisol, soon to be expanded to include immunoreactive insulin and glucagon. Likewise, hepatic ascorbate is being studied to determine to what extent MS-222 may be metabolized by the liver.

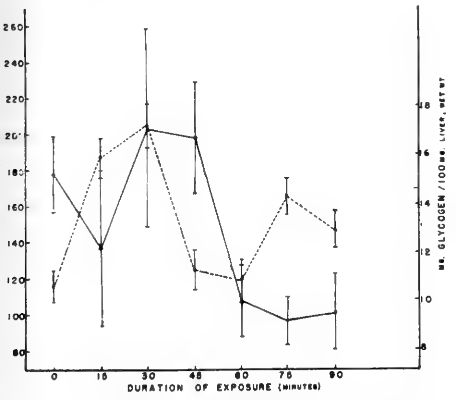


Figure 7.--Effect of duration of exposure to a 1:15,000 anesthetizing solution of MS-222 on rainbow trout blood glucose and liver glycogen concentrations. Vertical lines indicate standard error of the means. N=5 fish per group. Time "O" indicates values obtained for unanesthetized control fish. Glucose _______; Glycogen _______.

The chronic maintenance of trout possessing indwelling arterial and venous cannulae

Meaningful physiological data are often difficult to obtain by sampling blood of fish under conditions of acute stress. On the other hand, a fish maintained chronically with indwelling arterial and venous cannulae can be expected to be "more normal" in its physiological responsiveness after passage of an appropriate period of accommodation.

Several cannulation techniques were tried, some novel and some based on published works. None was satisfactory for the purposes intended. During a recent visit to our laboratory, Dr. Walter F. Garey of the Scripps Institution of Oceanography demonstrated a technique of chronic vascular cannulation which he found successful in respiratory and cardiovascular studies of nine fresh and salt water teleost species. This technique, now described in the literature (J. Appl. Physiol. 27(5):756-757 (1969)), is eminently satisfactory for arterial and venous cannulation of trout since extensive study has indicated:

(a) the dorsal aorta is the only feasible vessel for chronic maintenance of an indwelling arterial cannula because placement is rapid and effected with a minimum of trauma.

(b) the ventral aorta is the only feasible vessel for chronic maintenance of an indwelling venous cannula because all other venous vessels have proven too fragile to tolerate the operation.

Dr. Garey's technique recommends itself further since the simplicity of the operation makes possible its use in field studies.

Gerald J. Crowley

Modification of feeding behavior with stream experience

The time required for planted trout to begin utilizing natural food as efficiently as wild fish can be of considerable importance to their subsequent success. This is particularly true if they are introduced at times of decreasing food abundance, or face stiff competition from resident fish.

Up to now we have compared the stream feeding habits of hatchery rainbow trout that had lived in Convict Creek for 10 months with those of comparable individuals maintained for the same period in circular tanks on pelleted trout feed.

Although they fed on the same types of food as stream-acclimated fish, the "naive" tank fish as groups consumed fewer of each kind of prey. They also appeared considerably less apt than stream-acclimated fish to utilize caddis larvae in cases and surface-floating items (as opposed to mid-water drifting organisms). Apparently trout need to learn some new techniques before they can utilize these types of food efficiently. As yet we do not know the time necessary for such learning.

Quantitative comparison of night vs. day feeding in streams

As an important step in working out energy budgets for trout in streams, we undertook with Dr. C. R. Feldmeth of UCLA a study of rainbow trout feeding chronologies. Our method involved comparing the weight of prey consumed by rainbow trout during different 5-hour periods of the day and night. Similar studies were carried out in summer, autumn, and winter for seasonal comparisons.

On the basis of weight consumed, 5 hour feeding periods in both summer and autumn can be ranked in importance (from greatest to least): mid-day (9 AM - 2 PM); late afternoon and early evening (3 - 8 PM); middle of night (9 PM - 2 AM); and late night and early morning (3 - 8 AM).

The majority of prey taken in mid-day periods were terrestrial in origin, or the adult stages of aquatic insects. Together they constitute the surface-floating component of the organic drift. Second in importance were highl[y] mobile aquatic beetles, which our drift samplin[g] showed to be most active during the afternoon. Immature aquatic insects were rarely taken at mid-day, undoubtedly because most species enter the organic drift only during their nighttime activity periods.

In contrast to fish feeding at mid-day, night-feeding fish took numerous species of immature aquatic insects, and virtually no surface-drifting forms. The early morning study periods encompassed both darkness and daylight feeding, so trout consumed a mixture of surface-floating and aquatic forms. However feeding was light on both types of prey. The afternoon-evening periods also spanned both sunlight and nocturnal conditions, but both surface-floating and aquatic prey are more abundant at this transitional period, and feeding was more successful.

By December, when the reduction of surface-drifting forms could have made night feeding of prime importance, reduction in abundance of all types of drifting organisms made day-night comparisons impossible. Even wild brown trout from Convict Creek fed poorly in the study stream.

During mid-summer there was indication that light intensity on full-moon nights was sufficient to inhibit the activity of nocturnal aquatic insects, and thus reduce nighttime feeding success of trout. However, comparisons of full and new moon drift samples in October and December failed to show such a phenomenon.

Relationship between organic drift and trout feeding in high-altitude streams

We have found wild cutthroat or brook trout in virtually all streams accessible to populations in permanent waters. Some of the streams we have looked at are exceedingly small, and often temporary, but all contain aquatic organisms showing behavioral drift. For example, in a snow-melt rivulet discharging less than 0.08 cubic feet per second, we found that from 10 to 65 organisms drift past a given point hourly (mean 38 for 24 one-hour collections). Drifting organisms in the rivulet were most abundant in late afternoon and early evening, and least abundant just before dawn. This pattern was closely correlated with water temperature, which fluctuated as much as 12.5C° daily. The few resident brook trout appeared to be making a good living.

Although we have not observed feeding behavior directly, comparison of stomach contents of trout with the composition of organic drift in small streams suggests that drifting organisms make up most of their food. We have as yet found no system suitable to evaluate occurrence of organic drift as a factor limiting habitation by trout.

Post-planting movements of hatchery trout in a stream

We learned previously that rainbow trout planted singly or in groups disperse in a largely predictable manner, provided that methods of handling and introduction are the same. During summer, 1969, we looked at the effect on dispersal tendency of certain changes in these methods.

Results of a preliminary study on the effects of differing planting location on post-planting movements indicate that fish planted in riffle areas move predominantly upstream, whereas fish planted in pools tend to move downstream.

We also found that direction of dispersal from a riffle area can be controlled further by orienting all fish in a particular direction with respect to stream flow. When so oriented, rainbow trout tend to move in groups in the direction they enter the water.

In a study of the effects of night conditions on dispersal tendency, we found that dispersal rate at night is significantly greater than in the daytime. There was some evidence to suggest that night planting also leads to relatively fewer fish taking up residence in the vicinity of introduction. The time of day fish were planted did not seem to affect their direction of movement.

The effect of "hunger" on post-planting movements was studied by comparing fish fed to satiation before planting with fish deprived of food for 88 hours. Starved fish dispersed significantly faster than satiated fish, and might have dispersed to far greater distances had the study stream been longer.

Winter feeding of trout in high-altitude lakes

Since winter conditions of ice cover, cold water, and near-darkness last for 8 months or longer in high alpine lakes, they could have major impact on the ecology of lake inhabitants. In our first study (winter 1969-70) we are investigating feeding under the ice in two small, shallow brook trout lakes above 10,500 feet in the Rock Creek basin, and a similar lake containing Kamloops rainbow trout on a tributary of the East Walker River. Our aims in this study are to infer from the types of food eaten what the fish are doing at different times of the winter, and to infer from the condition of fish and the quantity of food they have eaten how much benefit they gain from their activities.

Our results to the end of this year indicate that trout are quite active in the winter and are still feeding on natural foods after two months under ice. Observations through holes suggest that fish in winter aggregate (and perhaps school) rather than remain solitary as in the summer.

By the end of December, fish of both species were in fairly good condition, and in many instances still had fat stored around their viscera.

Variability among individuals in their stomach contents is great, despite their apparent tendency to aggregate (Table 3). Most feeding involves bottom-living organisms and zooplankters, as opposed to the tendipedid pupae which predominate during the summer.

Our results to date suggest the following answer: at very low densities, the more aggressive fish defend largely inviolate territories, through which they move in a characteristic manner. Since even these individuals voluntarily restrict their activities to a small proportion of the pond area, there is room for the other fish to feed in portions exploited only by themselves.

Table 3.--Stomach contents of 10 brook trout taken in midwinter from Chicken Foot Pothole Lake (Elev. 10,761 feet). All were caught within 28 minutes through one hole in the 18-inch ice cover.

Fish No.	Numbers of organisms in stomachs			
	Diptera larvae	Water mites	Bivalve molluscs	Copepods
1	32	0	0	0
2	4	0	0	0
3	11	11	0	0
4	34	2	1	0
5	32	0	2	0
6	1	0	0	71
7	16	0	6	0
8	2	0	0	0
9	95	1	1	0
10	4	0	2	0

Although we have made a successful start on this project, its completion through this winter and on to spring thaw is threatened by the inadequacy of our transportation capabilities. We are arranging to test larger, more powerful over-snow machines; the most informative test conditions will likely not be encountered until February 1970.

Behavior of trout in experimental ponds

To understand the role of behavior in the ecology of trout, we have broadened observations to include four experimental ponds with natural food supplies. The ponds measure approximately 10 x 25 meters by 1 meter deep, and are equipped with screened inflow pipes and standpipe drains to maintain a constant water level. Fish food production seems to occur more or less uniformly over the pond bottoms.

One of our prime questions is why increasing densities in some still-water habitats result in general stunting rather than the increase in size variability observed in some food-scarce experimental situations.

As densities of fish increase, aggression becomes progressively less effective in separating ranges of movement, and overlapping use of water increases. In fact, the average water volume sought by individuals appears to be constant over large ranges of density. By virtue of this "shared" use of water and its associated food organisms, a fish decreases the number of prey encountered per unit distance traveled both for itself and for other fish using the same area.

Summer and autumn supplies of food in our ponds are very rich, consisting primarily of immature insects of the dipteran family Tendipedidae. Trout feed especially heavily on the pupae and emerging adults of this family. Aquatic beetles and Hemiptera bugs are also cropped at high rates.

With the advent of ice cover in the winter we developed ice-free standpipe drains, and plan to continue monthly stomach sampling through the winter. The first two under-ice samples indicated decreasing food consumption, as we might expect from the loss of midge pupae and emerging stages due to ice cover. As of the year's end, the fish were still maintaining their physical condition, presumably due to decreased maintenance costs in the cold water (Fig. 8).

Figure 8.--Rainbow trout are removed from one of the ponds for study of under-ice feeding.

As in alpine lakes, the fish in our ponds appear to feed in groups rather than solitarily. This is a striking change from summer behavior, whereby each fish has a distinctive pattern of movement.

Reproductive migrations of rainbow trout

Once again we finished a calendar year without trapping facilities on Convict Creek, but were able to determine timing of the spring run of rainbow trout by weekly electrofishing. The first spawners were encountered on 4 May, whereas the run in 1968 began prior to 7 March. This is a large difference in timing; the runs in both years coincided rather closely with break-up of ice cover on the lake.

Night behavior of trout

After several attempts, we abandoned the idea of pinpointing night positions of trout in streams by flash photography. Attenuation of the flashes is rapid, resulting in a dazzling cone of light for a short distance, and a relatively small strip of adequate visibility on the photographic plate. With luck, fish can be spotted by their shadows, but flashes in short succession indicated that the first flash, though only of 1/1200 second duration, drives them into hiding or to other stream areas.

Evaluation of an intramuscular tag for trout behavior studies

Observations of fish in our experimental ponds indicated that distances are too great and movements too frequent for identification of individuals with heat brands. We therefore tested the adequacy of a plastic intramuscular tag (Floy Tag Co.) which can be inserted rapidly with a mechanical apparatus.

The tag is a filament of plastic with a "T" on one end. By means of a hollow needle, the T is bent parallel to the filament and inserted into the opposite side through dorsal musculature and between interneural bones. When the needle is withdrawn, the T opens and hangs up on the interneurals.

After extensive tests in the ponds and in the Convict Creek controlled stream sections, we concluded that these tags cause no mortality of hatchery rainbow trout, and have no effect on feeding or other behavior. However, the flags originally provided on the protruding filaments had to be removed, as their presence resulted in significant tag loss.

The colored tags worked well for identification of fish in the experimental ponds, and they projected enough to be visible from an observation tower on either side. There was no tendency for fish to nip at, or otherwise react to, tags on other fish. We consider them suitable for behavioral observations requiring long-term retention and ready identification from a distance.

Thomas M. Jenkins, Jr.

Figure 9.--Laboratory residence and surroundings in March, 1969.

WARMWATER FISH CULTURAL RESEARCH LABORATORIES

Stuttgart, Arkansas
Kermit E. Sneed, Director

Laboratories

FISH FARMING EXPERIMENTAL STATION
Stuttgart, Arkansas
Fred P. Meyer, Chief

SOUTHEASTERN FISH CULTURAL LABORATORY
Marion, Alabama
Harry K. Dupree, Chief

FISH FARMING DEVELOPMENT CENTER
Rowher (Kelso), Arkansas
John J. Guidice, Chief

HIGHLIGHTS

U. S. acreages devoted to warmwater fish farming approached 40,000 acres. Estimated returns to the farmers for 1969 were $33 million.

Raceway culture at Stuttgart is producing 2,000 lbs. of fish in an area 100 ft. long, 15 ft. wide, and receiving a flow of 550 gpm.

Vitamin A acetate requirement for channel catfish was tentatively established at 1,000 to 2,000 units per kilogram of feed.

Channel catfish weight gains and dietary lipid levels were linear; best growth was from diets with 15 percent fish oil.

Weight gains of channel catfish fed purified diets with an insulin-sparing drug were greater than those of fish fed identical diets but without the drug.

Raw and pasteurized fish processing wastes have proved suitable as feed for catfish fingerlings.

Channel catfish have a significant growth response to increased protein percentage in feed and to feed amount.

Seasonal use of "demand" feeders shows that catfish use much more feed during early summer than is presently recommended.

Stocking density stresses can be partially compensated by supplying more feed.

Pre-determined feeding schedules were shown to have gross errors but could be used to produce an average fish crop.

Outcross hybrid catfishes failed to retain the high degree of vigor demonstrated by F_1 hybrids.

The white catfish x channel catfish hybrid is fertile and will reproduce.

Adult male channel catfish are more susceptible to quinaldine than adult females.

A trapping device using feed as a lure was successful in removing 90 percent of the fish in two attempts.

Fingerling catfish can be hauled at the rate of 2 lbs. per gallon in aerated well water at 55° F.

The S_{20}, W value, subunit structure, amino acid composition, and peptide maps of the Ig M immunoglobulins of paddlefish and longnose gar have been determined, and limited primary sequence data from the terminal end of the polypeptide chains shows a structural pattern similar to those of man.

Peak periods of disease incidence during a 5-year period were identified and corrective procedures suggested.

Aureomycin, sulfamethazine, and a combination of the two failed to protect fish from Aeromonas liquefaciens when included in the diet at low levels.

Branchiomyces sanguinis was identified in gill tissue of striped bass received from two locations.

Dalapon 24-hour LC_{50} value was determined to be in excess of 4,000 ppm at 42° F. for bluegills.

PAST, PRESENT, AND FUTURE STATUS OF FISH FARMING RESEARCH

Attempts by the U. S. Fish Commission to produce the channel catfish for sport and food began before 1892. In that year 1,300 fingerlings were produced in small ponds in Washington, D. C., from broodstock obtained from the Federal fish station at Neosho, Missouri. Considerable unrewarded effort followed this initial "success," but it was not until about 1915 that the Commission's Biological Station at Fairport, Iowa, gained useful insight into the habits and spawning requirements of this desirable species.

At about the same time, some states also began to rear channel catfish, notably Kansas, Oklahoma, Missouri, and Texas. By the 1930's production methods were fairly well defined and reasonably successful for the production of at least hundreds of thousands of fingerlings for stocking public fishing waters.

Among southern and southwestern fishermen, the channel catfish was on par as sport and food with the trout of northern waters. However, catfish were abundant in the streams, rivers, and lakes of its natural range, which undoubtedly reduced the demand for stocking. In the 1930's and 40's, following the construction of large numbers of man-made lakes and ponds, the demand for artificial stocking suddenly increased for largemouth bass, bluegills, and channel catfish. By 1950, there was a growing interest also on the part of irrigation farmers in Arkansas, Mississippi, and Louisiana to fish-farm abandoned land or large irrigation reservoirs. The buffalofish or carp used for this purpose did not produce a profit because of low consumer acceptance and low selling prices due to seasonal competition from wild fish which came to market simultaneously with farm-reared fish. Knowledgeable farmers realized that the high-quality, high-priced channel catfish might succeed where the buffalo and carp failed.

Two interests, potential fish farmers and sport fishermen, encouraged the Fish and Wildlife Service and a few institutions, notably Auburn University, to begin channel catfish research projects in the middle 1950's. This early research led not only to profitable catfish farming but increased production for public waters from both National and State fish hatcheries. The growth of catfish farming, particularly, has been so striking and captivating, with its promise of profits and public good, that many institutions and agencies are now engaged in some type of catfish research or testing program.

Research of the different agencies covers a broad spectrum of subjects, including basic nutrition, practical diets, stocking and feeding rates, harvesting, spawning, behavior, cage and raceway culture, silo culture, hybridization, and disease.

The Warmwater Fish Cultural Laboratories pursue a broad range of research associated with fish culture, especially catfish and baitfish farming. The Bureau of Commercial Fisheries is engaged in harvesting research at their Gear Research Station, Rowher (Kelso), Arkansas. They also do surveys and research concerned with processing and marketing farm-raised catfish.

Other agencies, mostly universities, are also engaged in catfish research, done mostly by graduate students who are usually supported by Federal money or a combination of Federal and State funds. About a half-million dollars are being spent by the states on channel catfish or baitfish projects, 75 percent of which is furnished by Federal funds under P. L. 88-309. The projects cover cage culture (State College of Arkansas and Southern Illinois University), nutrition and physiology (University of Georgia), behavior of catfish and other species when confined together (Illinois Natural History Survey), rearing in flow-through water systems (Skidmore Institute, Georgia) and rearing baitfish in the desert Southwest (Nevada Southern).

The Bureau of Sport Fisheries and Wildlife also conducts fish culture research through its Cooperative Fishery Units located at selected universities, particularly Auburn, Louisiana State, and Oklahoma State.

Kermit E. Sneed

NUTRITION

Demand feeders

Two 0.25 acre ponds were stocked at the rate of 1,600 per acre with blue and channel catfishes. In one pond the fish were fed by a demand feeder; in the other they were hand-fed 3 percent of body weight daily. Results are shown in Table 1.

Blue catfish growth was not significantly changed, but channel catfish grew better when fed by the demand feeder (Figure 1).

Figure 1.--Demand feeder similar to those currently used on catfish farms.

Table 1.--Data on growth and feed conversion for hand-fed and demand-fed blue and channel catfish.

	Hand-fed		Demand-fed	
	Blue	Channel	Blue	Channel
Stocking weight, grams	12	11	12	11
Harvest weight, grams	290	320	310	392
Gain, grams	278	309	298	381
Net production, pounds	123	136	131	168
Feed used, pounds	387		438	
Conversion	1.50		1.47	

A demand feeder was installed for demonstration purposes on a 1.0 acre pond stocked with 5,000 small channel catfish fingerlings. Starting from an average weight of 5.5 grams, these fish reached 272 grams, using 5,469 pounds of feed in 180 days. The entire pond produced 3,160 pounds of fish. An event recorder wired to a switch in the down-spout of this feeder showed almost constant use. Table 2 presents data calculated from the production and feed consumed. The high values for "Feed % Fish Wt." during the early part of the growing season indicates that the fish took more than one feeding each day. Conversion was satisfactory, but not as good as for other (hand-fed) ponds at the station. In August, Fintrol added to this pond killed an estimated 400 pounds of sunfish and shad. Uniformity of size was noticeably greater than that of fish in an adjoining 1.0-acre pond stocked with 2,700 fingerlings hand-fed once daily.

and conversion. Table 3 shows data for all tests.

Fish growing on feeds of different protein percentages and at different levels of body weight were analyzed statistically. A significant linear response was found for percent protein and feed amount, the equation using these two variables being:

$$y = 136.8 + 12.7\left(\frac{\%\text{ Protein} - 30}{5}\right) + 32.9\left(\frac{\%\text{ Feed} - 4}{2}\right),$$

with "y" expressed as pounds gain per 1,000 fish during the 70-day test period. An excellent fit for the data was also found in the quadratic equation:

Table 2.--Production in a 1-acre pond stocked with 5,000 catfish fingerlings using demand feeder.

Time days	Cumulative lbs. feed	Production lbs. fish	Conversion feed/gain	Feed % fish wt.
0	0	67	--	--
35	450	--	--	--
55	674	320	2.66	8
85	1,933	800	2.64	5.5
115	2,751	1,700	1.68	2.0
157	3,850	2,440	1.58	1.7
175	4,870	3,000	1.66	1.5
190	5,469	3,160	1.77	1.3

Percent protein and feed amount

Two short-term tests used varied percentages of protein in feed. One test at Kelso in 10'-diameter plastic pools combined the effect of protein percentage in feed (25 percent, 30 percent, and 35 percent) with the amount of feed used daily (calculated to be 2 percent, 4 percent, and 6 percent of fish weight). Pools were stocked with 50 18-gram channel catfish fingerlings, and each variation in protein and feed amount was tested in triplicate. At the same time, other feeds containing fish processing waste and fish meals were tested for growth

$$y = 139.4 + 1.30\,(\text{Protein Wt.} - 48) - 0.012\,(\text{Protein Wt.} - 48)^2,$$

"Protein Wt." being protein fed, which is the calculated amount from percent protein in feed times the amount of feed, again on a "per 1,000 fish" basis.

The slope of the regression line describing observed data from the pools is 1.2, indicating that for each percent increase in protein, 1.2 grams gain per fish will result.

Table 3.--Average final weight of catfish fingerlings for 70-day test in pools at Kelso.

Feed treatment		Fish weight (grams)	Conversion
25% Protein fed at	2%	61	0.88
	4%	78	1.23
	6%	82	1.73
30% Protein fed at	2%	65	0.76
	4%	86	1.09
	6%	92.5	1.48
35% Protein fed at	2%	68.5	0.74
	4%	88	1.05
	6%	97	1.37
Standard formula (30% Protein) using menhaden meal		93	0.78
Standard formula using catfish processing waste (heads, viscera, skins) dried into a fish meal		84	0.77
Commercial fish feed, 25% Protein		78.5	1.04
Raw catfish processing waste		98	2.4
Pasteurized catfish processing waste		101	2.4
Raw waste plus CMC as a binder		94	2.4
Raw waste using a feed meal as a binder		95	1.3

Table 4.--Biological and economic evaluation of feeds containing three levels of protein, fed to channel catfish in ponds during a 130-day period.

Amount of Protein	W_o	W_n	Conversion	Pounds gained /1000 fish	Net value
25%	21.7	181	1.82	352	$ 113.80
30%	21.6	198	1.65	389	126.20
35%	21.3	209	1.55	414	133.80

In 0.25-acre ponds at Stuttgart, production and feed conversion for feeds containing 3 levels of protein are shown in Table 4.

We found a significant linear response to increased amounts of protein in feeds provided at the 3 percent rate, under pond conditions. The slope of the regression line for this was 4.0; for each 1 percent increase in protein, fish responded with 4 grams more gain during the 130-day period.

Stocking rate vs. feed amount

White catfish were used to test the effects of feed amount and stocking rate on production. Duplicate 0.25 acre ponds were stocked at three rates, 1,200, 2,000, and 4,000 per acre, and fed 2 percent, 4 percent, and 6 percent of calculated fish weight based on monthly samplings. The results are best described by a graph (Figure 2). At each stocking rate, increased amounts of feed produced more fish weight, except that during the last month before harvest, no gain occurred in the ponds stocked at 4,000 per acre and fed 6 percent of body weight. These ponds had received 7,440 pounds of feed per acre during the season, and water conditions were marginal for fish culture. Although no mortality occurred, the presence of fish at the surface for several mornings was evidence of an oxygen-related stress.

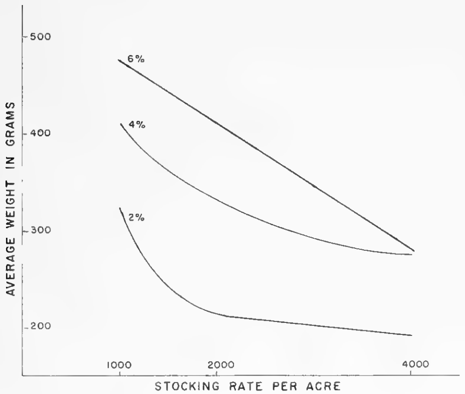


Figure 2.--Second-year growth response of white catfish stocked at three rates and fed at 2%, 4%, and 6% of body weight.

Economically, as shown in Figure 3, the 4 percent level of feeding proved best. Competition for feed required that more than 2 percent be used to assure that each fish received a portion. At 6 percent there was obviously an excess at each feeding.

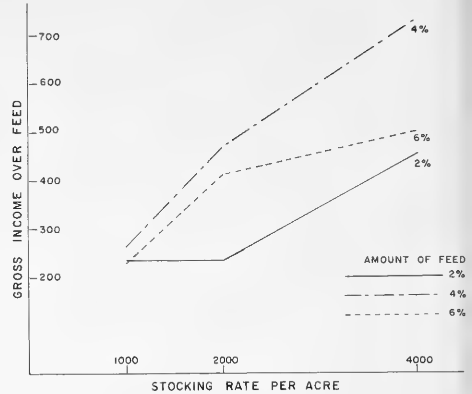


Figure 3.--Fish value minus feed cost for three stocking rates of white catfish fed at 2%, 4%, and 6% of body weight.

Basket culture

In late June, three wire cages were floated in a 1.0-acre pond. One basket contained 1,030 fish, another 500, and a third 200. These were fed a hard sinking pellet by hand until no feeding activity was noticed. Survival was excellent and growth was fair. Fish gained from 10 grams to 115 grams in 100 days. Average weights were similar in all cages.

Waldon H. Hastings

Dietary requirement of vitamin A acetate and beta carotene

In a previous study we indirectly demonstrated that channel catfish require the A vitamin, and we suggested this species could metabolize little of the A provitamin, beta carotene, to the vitamin itself. Vitamin supplementation of rations for pond-rearing of catfish is increasing and, thus, we need information on vitamin requirements and deficiency symptoms.

Our studies were in 32 glass aquariums supplied with a continuous flow of food-free well water. Water temperature during the first 18 months was a constant 70° F. and during the last 5 months ranged from 82 to 85° F.

For the first 13 months, the fish were exposed to low-intensity light (less than 25 Weston units at the water surface) during working hours, and to outside light and darkness the remaining hours. For the last 10 months of the experiment, the fish were exposed continuously to approximately 300 Weston units emitted from daylight-type fluorescent lamps. Light intensity was measured by holding a Weston photography meter at the water surface with the photocell perpendicular to the light source.

Each aquarium was stocked with 50 grams (approximately 20 individuals) of 4-month-old channel catfish on 27 September 1967. Fish were offered a series of purified diets that contained (in parts): vitamin-free casein - 33, white dextrin - 20, refined cottonseed oil - 10, carboxymethyl cellulose - 5, mineral mixture - 5, vitamin A-free vitamin mixture (in dextrose) - 1, and cellulose flour (dietary bulk) - 26. The 16 diets were supplemented with 0, 500, 1,000, 2,000, 3,000, 5,000 and 10,000 or 20,000 units of vitamin A as the acetate or as beta carotene per kilogram of dry ingredients. One hundred parts of the dry ingredients were mixed with 150 parts of water using the carboxymethyl cellulose as a binder, and stored at -10° C. until fed.

Feed allowances (dry weight basis) for all aquariums of fish were calculated on 2 percent body weight per day and provided 5 days each week for the first year of the experiment, and at the rate of approximately 1 percent for the remaining time. Feed allowances were revised quarterly based on the heaviest aquarium of fish.

For diets with different levels of beta carotene, weight gain was generally linear with provitamin levels through 20,000 units. Weight gain with diets that contained 20,000 units did not equal the gain with 1,000 and 2,000 units of vitamin A acetate, suggesting that even 20,000 units of beta carotene did not supply the needs of the fish (Figure 4).

Deficiency symptoms for fish fed the lower levels of both vitamin A acetate and beta carotene included reduced weight gain, protruding and opaque eyes, accumulation of clear serous

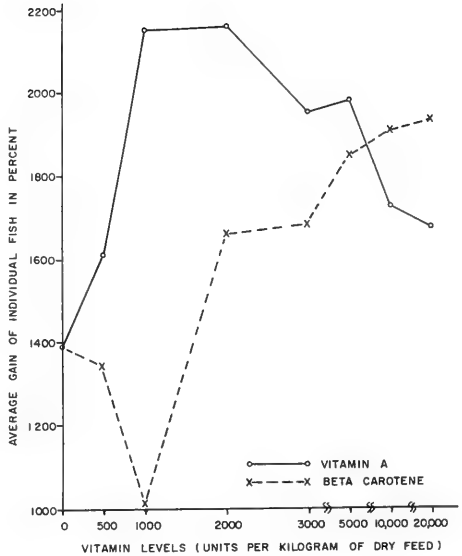


Figure 4.--Weight gain of channel catfish fed purified diets containing vitamin A acetate and beta carotene.

fluid in the body cavity, and death. Also associated with these symptoms was the presence of an eye-pupil-sized white spot on the epidermis between the eyes (pinel body). Lethargy was observed, but we do not know whether it was due directly to the deficiency or to a failure of the fish to feed.

The poor growth rate and relatively long time required to produce obvious deficiency symptoms needs explanation. Growth was slow during the first 18 months of the experiment due to low (70° F.) water temperatures, but increased to near normal rates during the last 5 months, when water temperature was increased to 82 - 85° F. With mammals, and probably with fish, vitamin A requirement is linked to animal size and not to metabolic rate, thus slow growth retarded the development of symptoms.

The fish were maintained in semi-darkness for the first 13 months. Since vitamin A is an essential component in the visual enzyme system (rhodopsin) and some vitamin A is destroyed each time rhodopsin is reduced to retinene and vitamin A, it appears reasonable that "darkness" would spare vitamin A.

Effect of fish oil and corn oil on growth and flesh quality of channel catfish

A need for low-cost rations for channel catfish production, combined with the availability of large quantities of oil from the manufacturers of fish meal, brought about a cooperative experiment between the Bureau of Commercial Fisheries' Technological Laboratory in Seattle, the Bureau of Sport Fisheries and Wildlife's Western Fish Nutrition Laboratory at Cook, Washington, and the Southeastern Fish Cultural Laboratory. Most practical rations currently used contain approximately 6 percent crude fat, and since many animals can utilize fat levels above 10 percent, we theorized that channel catfish could utilize more fat and thus spare the more expensive protein for growth.

The growth phase of the experiment was conducted in 75 glass-fronted aquariums each 2 x 2 x 1 foot deep and supplied with 0.2 or 0.5 gpm of 82-85° F. heated well water; the greater flow was used toward the latter part of the experiment. Each aquarium was stocked with 150 grams of 8-month-old catfish (about 20) and fed one of 15 purified diets that contained (in parts): hot-alcohol-extracted, micropulverized casein (ether extractable fat-maximum 0.01 percent) - 27.2, white dextrin - 15, mineral mixture - 5, vitamin mixture (in dextrose) - 3, and carboxymethylcellulose (diet binder) - 5. In 6 diets, 0, 5, 8, 12, 15, or 20 parts of bleached fish oil was added; in another 6 diets a like amount of corn oil was used; and the remaining 3 diets were supplemented with 12 parts of bleached fish oil with vitamin E (alpha tocopherol) as an antioxidant; 12 parts of the same bleached fish oil, but without the antioxidant; or 12 parts of "crude" (unbleached) fish oil. In each diet an amount of cellulose flour was added, ranging from 29.8 to 44.8 parts calculated to total each ration to 100 parts. All rations were stored frozen until fed.

Fish in all aquariums were fed equal amounts of feed daily, 6 days each week, and were weighed at the end of each 2-week period.

The influence of fish oil and corn oil on growth and flesh quality was based on weight gain and feed conversion. Analyses included liver glycogen levels, proximate analysis of the whole fish and the fillets, gas chromatographic identification and quantitation of stored fatty acids, separation and quantitation of metabolically essential lipids and the non-essential depot lipids, liver histology, and taste panel evaluation. All results on proximate analyses and fish taste have not yet been obtained, but sufficient information is available to summarize the experimental results and conclusions.

Weight gain increased as the level of bleached fish oil was elevated from 0 to 15 percent of the dry diet, but gain decreased at 20 percent (Figure 5). The oil used in the preparation of these diets did not contain an antioxidant, but the diets were stored near 10° F., which retarded increase in TBA value.

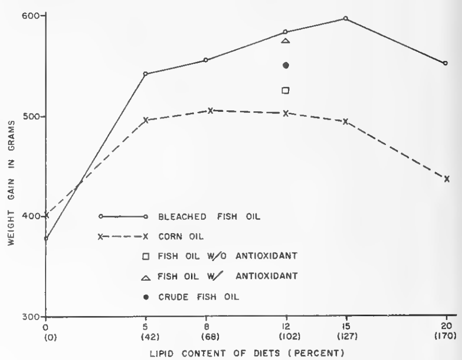


Figure 5.--Weight gain of channel catfish fed rations containing 0, 5, 8, 15, and 20 percent fish oil and corn oil. Numbers in parentheses represent grams of lipid offered during the experiment.

An antioxidant, alpha tocopherol, added at the level of 0.01 percent, appeared to retard increase in TBA value of bleached fish oil stored at room temperature. Gain of fish fed the tocopherol-protected oil was superior to the gain of fish fed unprotected oil, and approached that of the gain of fish fed freezer-stored oil (Figure 5). Gains of fish fed corn oil diets were inferior to those fed fish oil diets. The shape of the growth curve of the corn oil-fed fish, as compared with the curve for the fish oil-fed fish, demonstrates little benefit from corn oil. This may be due to a deficiency of essential fatty acids (C18 $\triangle$ 3).

The BCF Technological Laboratory reports the lipid, protein, ash, and moisture content of the fillets of fish fed the 0-15 percent fish oil diets were approximately the same for each diet oil level, although oil increased and protein decreased slightly with higher oil-containing diets. On the basis of protein and lipid content, fillets from the fish fed 20 percent fish oil diets were inferior to those fed 0 to 12 percent fish oil diets.

Taste panel tests at Seattle describe the flavor of the pond-reared (control) fish and the corn oil-fed fish as rather "bland" and "delicate". All the fish oil-fed fish acquired the odor and flavor of fish oil, and probably would not be acceptable to the public. In subsequent taste comparison sessions in which the skin was removed from the fish before cooking, the flavor of the fish oil-fed fish was greatly improved and possibly would be acceptable.

Researchers at the Western Fish Nutrition Laboratory report "There was no significant difference in liver glycogen content in any of the fish oil or corn oil-diet groups. Liver glycogen varied from 1 plus to 3 plus on a basis of 3 plus for maximum glycogen content. Ceroid appeared in small amounts at or near portal triads in most livers but was insignificant in amount. Sections were stained with hematoxylin and eosin for routine histopathological survey and other sections from each liver were stained with Best's carmine or with periodic acid Schiff (PAS) for glycogen. Representative samples from each diet group were also digested with salivary diastase for 20 minutes at room temperature, after which they were given the PAS reaction. These samples lost most of their PAS-positive material by diastase digestion, indicating that most of the PAS-positive material was indeed glycogen.

"Several liver samples from 3rd generation Donaldson rainbow trout were also stained with Best's carmine and with PAS, and all samples gave results for glycogen almost identical to results for catfish. An occasional cluster of plump, rounded liver vacuoles (which were probably fat vacuoles) was seen in catfish but their occurrence was so trivial and infrequent as to be considered negligible."

Harry K. Dupree

PHYSIOLOGY

Quinaldine

During catfish hybridization experiments, quinaldine was used to anesthetize fish during handling for hormone injection. Male fish were more susceptible than females. Responses observed consisted of loss of equilibrium and ultimate immobility.

Attempts were made to sex 6-inch channel catfish fingerlings following anesthetization with 10 ppm quinaldine at 66° F., but no association could be found between the sex of fish of this size and their drug response.

Dewey L. Tackett

Oxygen requirements of catfishes

The oxygen consumption of 16 catfish was measured with a respirometer (Figure 6). Oxygen was consumed at the rate of 0.48 mg O_2/g of body wt/hr. at 68° F. by 10-gram channel catfish. A 523-gram channel catfish consumed oxygen at the rate of 0.07 mg O_2/g of body wt/hr. at the same temperature.

Containers used in conjunction with the degassing system were changed from 3-gallon aquaria to economical 1-gallon pickle jars, but results from the 3-gallon aquaria could not be reproduced with the smaller vessels. We believe that the reaeration capacity of the larger container is greater than that of the smaller one due to a 4-fold difference in surface areas.

Dewey L. Tackett and John J. Giudice

Salinity tolerances of catfish hybrids

Various hybrids of channel catfish and blue catfish survived 96-hour exposures to 14-15 ppt salinity. Channel catfish and hybrid fingerlings (blue catfish x blue-channel) tolerated 14 ppt salinity for two weeks.

Immunology of warmwater fish

Cooperative studies with R. T. Acton, P. F. Weinheimer, E. E. Evans, D. Legler, and J. C. Bennett of the University of Alabama Medical Center in Birmingham, revealed that the paddlefish and gar, although responding well to a variety of antigens, only synthesize one type of immunoglobulin. This can be defined as a macroglobulin having a sedimentation coefficient of about 19S and a molecular weight of about 900,000. Catfish appear to respond somewhat more slowly but tentative evidence suggests synthesis of more than one type of immunoglobulin as far as physical and chemical properties are concerned. However, longer periods of observation will be necessary, using different antigens and different immunization schedules, before this can be clarified. The immunoglobulins of these fish represent about 40 percent of the total serum proteins.

Attention has also been directed toward the physical-chemical properties and subunit structures of the Ig M class of immunoglobulins, which have been isolated and characterized from the paddlefish and longnose gar. General structural relation exist among all vertebrate Ig M immunoglobulins observed, including those of man. The S_{20}, W values, subunit structure, amino acid composition and peptide maps of the immunoglobulin have been determined and evaluated, and limited primary sequence data from the amino terminal end of the polypeptide chains show a structural pattern similar to that of man.

The study should not only shed light on the genetics and biological function of immunoglobulins, which will be of value in trying to control the synthesis of these molecules in man, but also aid in the control, management, and development of fish which are more immunologically competent. It is also likely that once the structures of immunoglobulins (sequence of amino acids which make up the polypeptide chains) are known, various genetic markers may be present which can be used to select for other characters in breeding programs. Once the immunoglobulins are purified from an animal, we can look for the exact time during the animal's development, from egg to adult, when immunological maturation occurs. This will be valuable information and could aid fish culturists in determining the time when fingerlings may be safely introduced to new areas where new disease agents might be present. By correlating biological properties of immunoglobulins with structure, much information should be gained about the genetics, synthesis, and control of immunoglobulins at the molecular level.

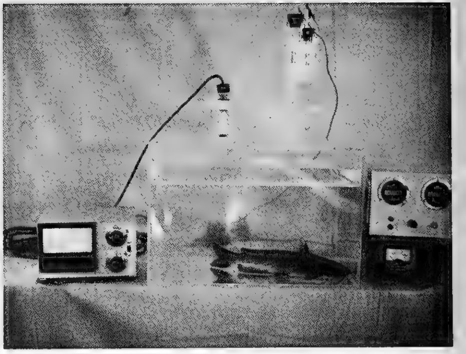


Figure 6.--Respirometer used in determining oxygen requirements of catfish.

Effect of an insulin extender and dextrin level on weight gain and serum glucose of channel catfish

A study was conducted on the use of an oral hypoglycemic agent to increase carbohydrate utilization in channel catfish. The drug employed was Tolinase R (Tolazamide), whose apparent mode of action is stimulation of the beta cells causing a release of endogenous insulin. An increased carbohydrate utilization may have a protein sparing effect.

Four levels of Tolazamide were incorporated in purified diets containing 4 levels of the carbohydrate, dextrin. Drug levels were selected on the basis of those used for diabetic control in humans. Forty-eight aquariums containing 100 grams of channel catfish fingerlings were fed for a total of 15 weeks. The fish were weighed at biweekly intervals throughout the experiment. At the termination of the experiment, blood glucose levels were measured after the fish had fasted 48 hours and at intervals of 1, 2, 4, 8, 18, and 24 hours after feeding.

Weight gains were generally higher for fish fed carbohydrate plus the drug. Without Tolazamide, there was no difference in weight gain between fish receiving 20 percent and 30 percent carbohydrate diets. Those fish receiving the 20 percent carbohydrate diet with 250 μg of Tolazamide per gm of dry feed exhibited the greatest gain. These also exhibited the most consistent blood glucose levels.

Blood glucose levels began to rise within one hour after feeding and reached their peaks at approximately 8 hours. Within 24 hours blood glucose values had returned to or near normal.

Blood glucose levels were consistently high for those fish receiving no carbohydrate as dextrin in the diet. This was contrary to the expected hypoglycemia. If comparisons can be made with diabetic animals, this is suggestive of gluconeogenesis and could account for the elevated blood sugar.

Harry K. Dupree

FISH CULTURE

Spawning catfish hybrids

Limited spawning attempts were made with hybrid catfishes at Marion. The channel catfish x white catfish hybrid that outperformed the parent species and other hybrid crosses when tested in aquariums was found to be fertile. Reproduction was accomplished by injecting human chorionic gonadotropin at the rates and with the techniques normally used for hormone-induced spawning of channel catfish. It was not determined conclusively that this hybrid will reproduce naturally under normal pond conditions, but the genitalia of female fish suggested that they were "spawned-out" when examined soon after the normal spawning season for white and channel catfishes.

O. L. Green

Hybridization

Growth of 6 groups of hybrid catfishes was compared to that of channel catfish. One-hundred grams of fish from each lot were placed in 30-gallon aquaria to which running water (75° F.) was supplied. All aquaria received equal amounts of feed daily.

Channel catfish lots were replicated 6 times, while the hybrid groups were replicated 3 times. The best performer in this study was the white catfish x blue-channel hybrid. Results show in Table 5.

Table 5.--Weight gains in channel catfish and six groups of hybrid catfishes fed equal amounts of the same diet in aquariums for 70 days.

Hybrid	Initial average weight (grams)	Final weight (grams)	Percent gain
Channel catfish	3.2	6.9	116
Blue-channel hybrid x channel catfish	3.3	8.7	157
White catfish x blue-channel hybrid	3.2	12.1	241
Blue-channel hybrid x blue catfish	5.3	16.4	207
Blue-channel hybrid x white catfish	11.6	35.7	150
Blue-channel hybrid x blue-channel hybrid	3.6	10.1	178
Blue catfish x channel catfish	8.3	15.7	69

The blue-channel hybrid x channel catfish grew faster than the channel catfish in this test, but the reverse was true in trough culture studies in which 1,150 fish of each group were held in separate indoor troughs having a water volume of 8.6 cubic feet. Each trough received 1 gpm of water at a temperature of 80° F. Commercial catfish pellets were offered to each group in equal amounts. Results appear in Table 6.

Both channel catfish and the white catfish outgrew the outcross hybrid even though the hybrid catfish was larger at the beginning of the test. In aquarium studies the cross outgrew the channel catfish.

Inconsistencies of our evaluation of growth of catfishes and their hybrids suggest a need for improvement of evaluation techniques. Knowledge of the variability of growth by different sibling lots should be a prerequisite to comparisons between fishes. Growth of sibling lots of the channel catfish should be compared to establish variability between lots. This variability between sibling lots of the same species may exceed the variability between sibling lots resulting from other species or hybrids, so no meaningful comparisons are possible.

Table 6.--Weight gains in channel catfish and in blue-channel x channel catfish in troughs.

Weight changes	Channel catfish	Hybrid catfish
Initial average weight (grams)	1.2	1.5
Average weight at 42 days (grams)	3.0	3.4
Percent gain in 42 days	145	132
Average weight at 130 days (grams)	8.5	9.1
Percent gain at 130 days	585	543
Average weight at 175 days (grams)	-	11.4
Percent gain at 175 days	-	667
Percent survival	74	77

Although the hybrids reached a larger size than the channel catfish, they gained at a slower rate as reflected by the percent gain. The size of the largest hybrid exceeded that of the largest channel catfish. It is noteworthy that 881 catfish were raised to a total weight of 10,000 grams (2.5 lbs per cubic ft. of space) under the conditions described above.

Another comparison of growth was made in 4 one-tenth-acre ponds. This study included equal numbers of channel catfish, white catfish, and the white catfish x blue-channel catfish. Table 7 has the results.

John J. Giudice

Table 7.--Growth of catfish and hybrids in ponds.

	Initial average weight (grams)	Final average weight (grams)
White catfish	32	536
Channel catfish	35	558
White catfish x blue-channel hybrid catfish	72	368

Feed Schedule

A predetermined feeding schedule was tested on four 0.1 acre-ponds, each of which contained 50 channel catfish (35 grams), 50 white catfish (32 grams), and 50 hybrid (blue-channel x white) catfish (72 grams). This test was made to study the feasibility of eliminating the burdensome and inaccurate task of periodic sampling of fish populations for the purpose of adjusting feeding schedules. Previous knowledge of the growth rates of channel catfish at given stocking rates provided the basis for setting the schedule. The schedule tested throughout one growing season was as follows:

Dates	Pounds per acre
April 15 to June 15	10
June 16 to August 31	20
September 1 to September 30	30
October 1 to November 1	40

Commercial catfish pellets containing 33 percent protein were given 5 days per week, weather permitting. No adverse conditions were observed during the study. The average conversion rate was 1.8 to 1.0 and the average production was 1,650 pounds per acre, of which 1,370 pounds were marketable (0.75 pounds or above). All channel and white catfish were saleable, whereas 90 percent of the hybrids were subsaleable. The fish were harvested on October 8 at the end of a 181-day growing season.

At the beginning of the test (April 10), all lots received feed considered equal to 6 percent of their body weight. Prior to the first scheduled increase in feed amount, a sampling of the fish indicated they had been given only 2 percent. The scheduled increase in the amount of feed resulted in their being given 4 percent. During this feeding period the rate dropped to 1 percent before the next scheduled increase (September 1). Sampling revealed that during September, 30 lbs/A feed was equivalent to approximately 2 percent of the body weight. On October 1, the feed amount was increased to 40 lbs/A as scheduled. The fish were harvested 8 days later and the actual feed given at that time amounted to only 2.7 percent of their body weight.

This study suggests that average production may be achieved by using a pre-determined feeding schedule based on knowledge of the growth of fish under the existing conditions. The amount of marketable fish, total production, and feed conversion at the end of the growing season were similar to those which could be expected from feeding the amounts of feed dictated by periodic sampling. Discrepancies observed during this test, however, indicate that further refinements of feeding schedules could greatly increase efficiency.

John J. Giudice and Dewey L. Tackett

Hauling

An experimental hauling unit was constructed with inside dimensions of 2 x 2 x 2 feet. Air was supplied through copper tubing placed at the bottom of the unit. This tubing was formed into a square 18 inches on a side and had 1/32-inch holes drilled 2 inches apart around its periphery.

The unit was filled to a depth of 6 inches with well water having a total alkalinity of 450 ppm and a pH of 7.4. This contained 2 cubic feet or 15 gallons with a surface area of 4 square feet. Air entered at the rate of 0.5 CFM. Dissolved oxygen was monitored continuously with a membrane-type electrode. The temperature remained at 63° F. throughout the study. Thirty-two 1-pound catfish were placed in the unit and after 30 hours were found to be in excellent condition. The dissolved oxygen level dropped to 40 percent of saturation 1 hour after the introduction of the fish. Thereafter there was a gradual decrease in the level of dissolved oxygen to 25 percent of saturation at 30 hours. The pH remained constant, suggesting there was no accumulation of ammonia or carbon dioxide.

These data suggest that with sufficient aeration in waters of high alkalinity, channel catfish can be transported at the rate of 2 pounds per gallon of water.

In response to a request from the State of California, tests were run to learn the number of catfish fingerlings which could be transported in a gallon of water. Lots of channel catfish were prepared which yielded loadings of 1.25, 1.5, 1.75, and 2.0 lbs. per gallon of well water. Compressed air was used for aeration. Temperature during the test was 72° F. ± 2°. Survival in all lots was good during the first 16 hours. However, it was noted that at the 2 lbs/gal. level, most of the fingerlings exhibited convulsions and died when removed from the container. Approximately 10 percent of those in the 1.75 lbs/gal. loading exhibited similar behavior. It was concluded that 1.5 lbs/gal. would represent a safe level at which to transport fingerlings at 72° using compressed air for aeration.

A shipment of blue catfish fingerlings was sent to California in December in milkcans filled with well water and aerated with chilled compressed air. Temperature during hauling was approximately 55° F. Nineteen cans were stocked at 1.5 lbs/gal., and one can was stocked at 2.0 lbs/gal. All lots had excellent survival during and following a 14-hour journey indicating that at 55°, 2.0 lbs/gal. may be a safe loading density.

Dewey L. Tackett

Fish transfers

During 1969, stocks of fish produced as an adjunct to research activities were transferred to State and Federal organizations. The Southeastern Fish Cultural Laboratory provided 180,378 catfish weighing 2,814 lbs. and the Fish Farming Experimental Station transferred 11,850 lbs. (26,300 fish).

Kermit E. Sneed

Combination stocking of channel, white, and blue catfishes

Researchers, fish farmers, and sportsmen have long discussed the possible desirability of stocking a combination of catfishes for sport and food. At Auburn University, researchers conducted tests with white catfish stocked alone and in combination with channel catfish in fish-out ponds. Little work has been conducted on combination stocking of fish for commercial food production and no controlled studies, to our knowledge, using blue catfish.

During the period of April 7-16, fish were individually weighed, measured, and stocked into triplicated 0.1-acre earthen ponds. Stocking rates are presented in Table 8. All ponds were fed equal amounts of feed each day, based on 3 percent of the calculated weight of the fish. Manufacturers' analysis of the ration is shown in Table 8.

The ponds were drained on Day 199 of the test, and the fish were individually weighed and measured. Survival of the channel catfish at all stocking rates ranged from 94 to 96 percent, exceeded somewhat the survival of the blue catfish (82-93 percent), and greatly exceeded the survival of the white catfish (10-80 percent). The data from Prather's work in which white catfish were stocked alone and in combination with channel catfish support these observations.

No statistical difference was measured between the total productions of any two stocking combinations. Average production ranged from 1,930 to 2,147 pounds per acre, a difference of only 11 percent, which is well within experiment variations observed in earthen ponds. Some differences in production did occur in the various stocking combinations, but inspection of the data in Table 8 suggests that the greatest production occurred in those ponds in which the greatest survival occurred.

The data in Table 8 also show that at all stocking ratios the weight contribution of channel catfish exceeded their stocked percentage. For example, channel catfish comprised 90, 80, and 50 percent of the stocked number, but their weight contribution to the total production averaged 95, 90, and 66 percent, respectively. Thus, it appears that best survival and best production was obtained in those ponds stocked with channel catfish alone or with a large percentage of channel catfish.

Table 8.--Growth and survival of channel, blue, and white catfishes stocked in combination in 0.1-acre ponds. Fish in each pond were offered 229 pounds of a floating-type ration[1] during the 199-day experiment period.

	Number stocked[2]			Percent harvested			Pounds harvested			Total Production/	
Pond	Channel	Blue	White	Channel	Blue	White	Channel	Blue	White	acre (pounds)	Factor[3]
1	180	10	10	99	90	10	225	8	0.8	2,338	1.0
5	180	10	10	88	90	10	198	7	0.9	2,059	1.1
9	180	10	10	97	100	10	193	10	1.0	2,040	1.1
	180	10	10	95	93	10	205	8	0.9	2,147	1.1
2	160	20	20	100	100	35	188	14	5	2,070	1.1
6	160	20	20	98	85	65	183	15	9	2,070	1.1
10	160	20	20	93	80	40	192	14	6	2,120	1.1
	160	20	20	94	88	47	188	14	7	2,087	1.1
3[4]	100	50	50	96	26	68	110	10	20	1,490	1.6
7	100	50	50	93	78	90	130	37	28	1,950	1.2
11	100	50	50	94	86	70	124	35	32	1,910	1.2
	100	50	50	94	82	80	127	36	30	1,930	1.2
4	200	55	55	98	--	--	214	--	--	2,140	1.1
8	200	--	--	97	--	--	208	--	--	2,080	1.1
12	200	--	--	92	--	--	209	--	--	2,090	1.1
	200			96			210			2,103	1.1

[1]Typical analysis (in percent) by manufacturer: crude protein not less than 32, crude fat not less than 2.5, and crude fiber not more than 8.

[2]Fish size, 35 per pound.

[3]"S" or supplemental food factor. Obtained by dividing pounds of food offered by the pounds of fish gain without regard to natural food present in the ponds.

[4]Results from Pond 3 not included in the averages due to the non-typical poor survival of blue catfish.

Individual weight and length measurements were made of the fish at the start and end of the experiment. Non-statistical graphic plots of most of the data show that the variation in weight of the harvested channel catfish exceeds by 100 percent the variation in weight of the stocked fish. However, a similar comparison for stocked and harvested blue catfish suggested a fairly uniform population growth rate. Low survival levels of the white catfish precluded any conclusion. Thus, we believe the data show that variation in the size of the harvested fish is due to differences in the sizes of the stocked fish and to differences in the growth rate potential or aggressiveness of individual fish. Sex differences were considered, but after sexing a reasonable sample of fish, we could not attribute all the variation to that factor.

O. L. Green

FISH BEHAVIOR

Trap sampler

Additional tests were run to evaluate the effectiveness of a trap for sampling channel catfish. Groups of 100 fingerlings were tested in a 60-gallon aquarium. The fish were allowed to move freely about the aquarium, but were fed inside a 1-foot-square trap. The trap was constructed of hardware cloth with one open side which could be closed by dropping a plexiglas gate. The fish were fed in the trap 65 times over a period of 1 month. Once every 5 days, after food was offered, fish in the device were trapped and removed from the aquarium. The device was most effective when placed directly on the aquarium bottom, trapping 99 percent of the fish in 2 trials, compared to 87 percent in 7 trials when placed 6 inches above the bottom. As a comparison, the trap was also tested with white catfish fingerlings. One hundred percent of the fish were removed from the aquarium in 1 trial after they had been fed in the device 30 times during a 10-day period.

A larger trap (4 feet by 6 feet) for tests with 8- to 16-inch channel catfish was installed on the bottom in a 1/40-acre pond. The fish were first trained to feed on floating pellets, and then food was offered inside the trap. After they had been fed in the device 38 times during a 13-day period, 90 percent of the fish were removed from the pond in two trials (Table 9). Trapped fish were not returned to the pond between tests. During this period surface water temperatures taken at midday ranged from 69 to 78° F.

Sonic attractant

Mechanical sounds associated with surface feeding activity of channel catfish were recorded with a Panasonic battery-powered recorder (Model RQ-102 S). Fifty 16-month-old fish were held in a 34-gallon aquarium. To avoid background noise in the recordings due to the operation of an aquarium aerator and pump, the fish were conditioned to associate the cessation of this noise with food, thus avoiding fright response which normally follows the sudden interruption of a continuous sound. Playing back feeding sounds to the fish that had produced them evoked a generalized exploratory response such as might result from any unrelated sound of a similar frequency and volume. However, these fish were easily trained to exhibit a conditioned response to their recorded feeding sounds by following these sounds with food. After 20 rewarded trials the fish showed active surface feeding behavior prior to the introduction of food.

Learning capacity

Tests were run to study learning in channel catfish. Twenty 17-month-old fish were held in a 60-gallon aquarium which could be partitioned into 2 equal sections by dropping a transparent plexiglas divider, thus allowing the fish in each section to be counted. For 34 days the fish were fed by introducing equal amounts of food simultaneously in both ends of the aquarium; they soon fed actively at both sites. They were then trained to associate the dropping of the plexiglas divider with the introduction of food. This was done by offering 4 food pellets in each feeding area simultaneously. After 15 seconds the divider was dropped, and 15 seconds later the regular amount of food was introduced in each area. Fish in each half of the aquarium were then counted, and after 2 minutes the divider was raised. At first, dropping the divider

Table 9.--Effectiveness of a trap in harvesting 16-month-old channel catfish in a 1/40-acre pond.

Test no.	No. of fish in pond	No. of fish trapped	Percent trapped (of fish in pond at time of test)	Percent left in pond (of original no. of fish)
1	318	238	75	25
2	80	47	59	10

caused a fright response, and the fish did not feed until after it was raised. However, after 12 reinforced trials some fish began feeding when the food was first offered, and the initial fright response gradually diminished. Soon they fed actively when the food was introduced.

After 100 conditioning presentations over a 32-day period, a new procedure was initiated in which the fish in only one half of the aquarium (section B) were rewarded. Results of these tests are shown in Table 10. Even prior to conditioning, section A of the aquarium was used as a sanctuary when the fish were distributed. This preference was evident once conditioning was begun, with an average of 67 percent of the fish choosing section A during the first 25 trials. As conditioning progressed the percentage of fish in section A decreased. After 100 reinforced trials, the fish in only section A decreased. Then, after 100 reinforced trials, the fish in only section B were rewarded, but the effect of this change was not apparent. In 50 additional trials the percentage of fish in section B was not significantly greater than in earlier trials.

Robert Tarrant

Table 10.--Distribution of 20 channel catfish in a 60-gallon aquarium in which they were trained to associate the dropping of an aquarium divider[1] with the introduction of food in both ends of the aquarium. After 100 reinforced trials, the fish in only one half of the aquarium (section B) were rewarded.

Trial no.	Mean percentage of fish in section A	Mean percentage of fish in section B
1 - 25	67.0	33.0
26 - 50	63.8	36.2
51 - 75	59.8	40.2
76 - 100	55.2	44.8
101 - 125	52.2	47.8
126 - 150	49.8	50.2

[1]Preceded by four food pellets in each end of the aquarium.

DISEASES AND PARASITES

Henneguya infections

A species of Henneguya which develops in the interlamellar spaces of channel catfish gills has been observed for a number of years. It was known from hatcheries at Marion, Alabama, and Tishomingo, Oklahoma, as well as from certain natural environments. Recently this parasite has appeared on an increasing number of private fish farms, perhaps due to interfarm shipments or to the use of wild fish as broodstock.

While most species of Henneguya do not cause problems of epizootic proportions, the species developing in the interlamellar spaces causes catastrophic losses. This form appears similar to Henneguya exilis but differs in two respects. The ends of polar filaments of the interlamellar form are coiled as in a corkscrew but are straight in H. exilis. Henneguya exilis develops within the blood capillaries of lamellae whereas the new form develops in basal cells between lamellae. (See Figures 7-10.)

Over 90 percent of the interlamellar space on fingerling channel catfish has been observed filled with cysts. Heavily infected fish show symptoms of anoxia, even in waters containing 8 ppm dissolved oxygen. Fingerling producers have reported losses of over 95 percent in fingerlings less than 2 weeks old. Post-larval fingerlings have been observed with every interlamellar space filled with a cyst.

Although farmers have been urged to destroy infected fish, this has not been done, and the spread from infected farms to new areas through sales has been documented.

Lernaea control studies

Dursban and Naled were compared with Dylox under pond conditions to evaluate their potential use in the control of the anchor parasite, Lernaea cyprinacea. Dursban at rates of 0.02 and 0.03 ppm proved overly toxic to the fish even though it did control the parasites; at 0.03 ppm it was lethal to the fish in both

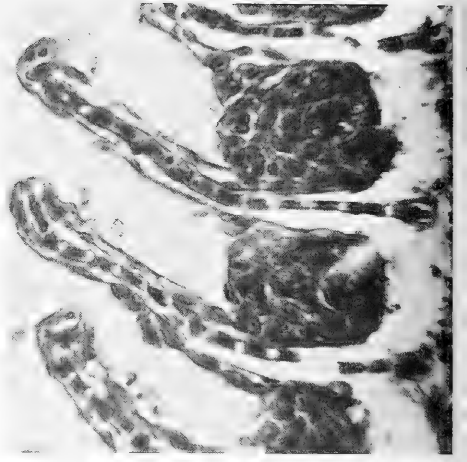


Figure 7.--Early development of interlamellar form of Henneguya sp. in gill tissue of channel catfish. Note Costia also on lamellae. (K. E. Sneed Photo)

Figure 8.--Later development of interlamellar form of Henneguya sp. in gill tissue of channel catfish showing complete filling of space between lamellae. Costia is also present. (K. E. Sneed Photo)

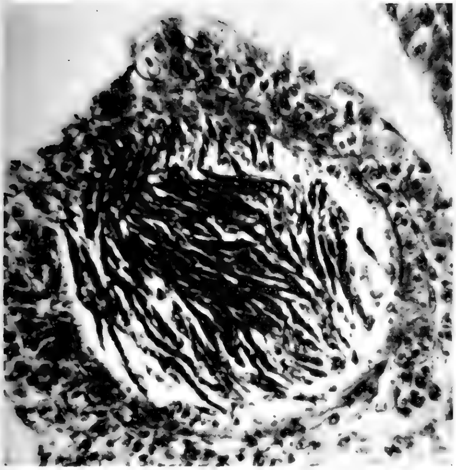


Figure 9.--Mature cyst of interlamellar form of Henneguya sp. showing spores of parasite.

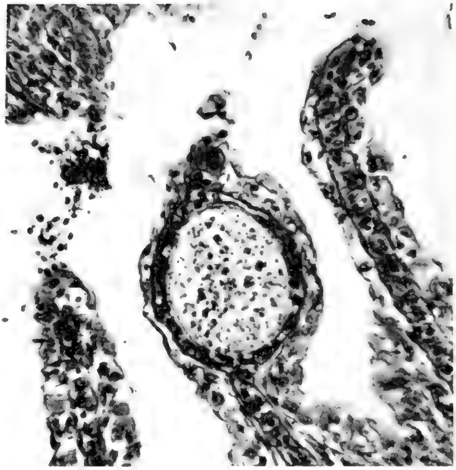


Figure 10.--A developing cyst of Henneguya exilis in channel catfish gill tissue. The cyst is within the lamellae rather than between, as in Figures 1-3. (K. E. Sneed Photo)

replicates. Some mortality occurred at 0.02 ppm and all fish surviving this level developed scoliosis and lordosis.

Naled is a compound widely used in Israel for the control of the anchor parasite. However, for the second successive year, it has failed to give control, even at levels 4 times that used in Israel. An exchange of chemical with Dr. Sarig has been achieved and the Israeli formulation will be tested at Stuttgart this year.

Branchiomyces sanguinis

Branchiomyces sanguinis was positively identified in the gills of striped bass, Morone saxatilis, received from two locations. This fungus is responsible for a disease known as "gill rot" in Europe, where it is considered to be a serious threat to commercial fish culture. Dr. Pietro Ghittino of Italy confirmed the diagnosis during his visit to the laboratory this fall. Histological sections are in preparation and a manuscript will be prepared when the studies are completed.

Prophylactic use of drugs

Aureomycin and Sulfamethazine were used continuously at low dosages in the daily ration of channel catfish confined in 10-foot-diameter plastic swimming pools to determine if these compounds had a preventive effect on the development of bacterial infections or a growth-stimulating effect on the fish. The fish experienced severe low-oxygen stresses on several occasions, but few fish were lost. Ten to 14 days after these stresses, however, Aeromonas liquefaciens infections appeared in treated and untreated lots, and all groups suffered nearly complete mortalities. (See Table 11.) Only 81 fish of the original 1,200 used in the experiment survived, and no benefits were noted in the prevention of bacterial disease. Although some increase in growth was apparent in the treated fish, it was not significant.

Fish disease symposium

Dr. Meyer assisted Dr. Snieszko by serving as a section chairman in the preparation of a

Table 11.--Survival of channel catfish receiving medicated rations following stresses associated with low levels of dissolved oxygen.[1] Experiment begun June 2, 1969.

Pools	Treatment	Numbers of dead fish, by day																				9/17 Survival
		August		September																		
		30	31	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	
#1	Chlortet.									1	7	29	53	4	2	2						0
#2	Comb.													1	6	42						51
#3	Comb.											2	5	21	56	7	2					7
#4	Control											1	4	7	16	62	10					0
#5	Sulmet											2	1	2	4	15	68	6				2
#6	Control								2	7	26	24	37									0
#7	Control								2	19	76											3
#8	Sulmet	1	2	35	62																	0
#9	Sulmet								4	23	63	4	1									4
#10	Comb.							2	8	19	58	9	0	1								2
#11	Chlortet.											2	9	21	53							12
#12	Chlortet.	Test terminated June 30 due to raccoon damage.------------------------------																				

[1]Low oxygen stresses occurred August 25 and September 2.

fish disease symposium for the American Fisheries Society.

Diagnostic service

During 1969, 475 cases were handled by the diagnostic laboratory at Stuttgart, and 125 by the Marion, Alabama, laboratory. The majority of these cases occurred from March 1 through October 31. Unseasonably hot, dry weather aggravated the fish by adding environmental stresses to bacterial or parasitic diseases. Oxygen depletions were common.

A distribution of incidence of types of etiological agents diagnoses is shown in Table 12. Pesticide-related problems were more numerous than during any previous year. Liver abnormalities believed to be related to inadequate nutrition also were apparent for the first time.

Fred P. Meyer

PESTICIDES

Dalapon toxicity

Toxicity tests, using new facilities, were conducted during the latter part of the year. Results with the herbicide Dalapon indicated 24-hour LC_{50} values in excess of 4,000 ppm at 72° for bluegills. Twenty-four-hour LC_{50} values at 93° with channel catfish were consistently near 3,000 ppm. No pronounced reduction in toxicity was noted at lower temperatures. Simultaneous testing under dark and light conditions indicated Dalapon to have a higher toxicity in total darkness. Light may, therefore, partially degrade Dalapon.

Toxicity also appears to be lower under alkaline conditions.

Varying results were obtained in attempts to raise cattails under artificial light in the laboratory. The results were generally satisfactory, and this technique may be used in tests in which cattails, water, soil, and bluegills, channel catfish, and other fish are exposed to ^{14}C-tagged herbicide in a controlled environment.

Ray L. Argyle

EXTENSION

Fish farming statistics

Fish farming statistics were revised and updated periodically during the year, using a cross-reference system of data cataloging. Current lists of fish farmers by States, species of fish reared, fish processing plants, haulers of live fish, dealers in fishery supplies, fish feed manufacturers, catfish franchise restaurants, and consulting personnel in fisheries are among the data maintained. We also tabulate acreages of fish farms in each State, by county, with records of growth of the industry in recent years (Table 13).

Over 4,800 requests for information were received at the Fish Farming Experimental Station in 1969, and in excess of 250 requests at the Southeastern Fish Cultural Laboratory.

Visitors at the Fish Farming Experimental Station in 1969 numbered over 1,400. One hundred eight were foreign visitors representing 28 countries. Ten students and researchers from 5 countries abroad spent a total of 68 days at the Stuttgart station to observe and study American fish farming.

Don S. Godwin

Table 12.--Numbers of various etiological agents encountered in disease cases handled by the diagnostic laboratory at the Fish Farming Experimental Station during 1969. (More than one organism was encountered in a number of cases.)

PROTOZOANS:	
Ichthyophthirius multifiliis	28
Trichodina sp.	172
Scyphidia sp.	129
Trichophrya ictaluri	26
Chilodonella sp.	12
Plistophora ovariae	27
Costia sp.	26
Myxosporidia (all species)	55
MONOGENETIC TREMATODES:	
Gyrodactylus elegans	28
Dactylogyrus sp. (all species)	21
Cleidodiscus sp. (all species)	101
DIGENETIC TREMATODES:	
Clinostomum marginatum	1
ENVIRONMENTAL PROBLEMS:	
Oxygen depletions	25
Pesticide-related problems	26
BACTERIAL DISEASES:	
Aeromonas liquefaciens	49
Pseudomonas sp.	12
Myxobacteria	48

Table 13.--Estimated gross income, by States, from intensive culture of warmwater fishes in 1967, 1968, and 1969

State	Species	1967 Surface acres	1967 Estimated income	1968 Surface acres	1968 Estimated income	1969 Surface acres	1969 Estimated income
Ala.	Minnows	$ 620	$ 190,960	$ 620	$ 190,960	$ 528	$ 187,440
	Catfish	100	47,000	496	233,120	1/ 933	438,510
	TOTAL	720	237,960	1,116	424,080	1,461	625,950
Ark.	Minnows	14,500	5,147,500	15,000	5,325,000	20,150	7,153,250
	Catfish	4,750	2,997,250	7,632	4,815,792	11,736	7,405,416
	TOTAL	19,250	8,144,750	22,632	10,140,792	31,886	14,558,666
Calif.	Minnows	-	-	-	-	160	56,800
	Catfish	-	-	-	-	587	275,890
	TOTAL	-	-	-	-	725	332,690
Fla.	Minnows	-	-	-	-	80	28,400
	Catfish	200	94,000	224	105,280	224	105,280
	TOTAL	200	94,000	224	105,280	304	133,680
Ga.	Minnows	-	-	-	-	-	-
	Catfish	200	94,000	699	328,530	2/ 699	328,530
	TOTAL	200	94,000	699	328,530	699	328,530
Kan.	Minnows	200	61,600	200	61,600	-	-
	Catfish	800	376,000	406	190,820	483	227,010
	TOTAL	1,000	437,600	606	252,420	483	227,010
La.	Minnows	1,500	462,000	2,300	708,400	1,971	705,705
	Catfish	1,500	705,000	2,693	1,265,710	3,222	1,514,340
	TOTAL	3,000	1,167,000	4,993	1,974,110	5,193	2,220,045
Miss.	Minnows	1,500	462,000	1,500	462,000	3,768	1,337,640
	Catfish	4,500	2,839,500	8,966	5,657,546	17,972	11,340,332
	TOTAL	6,000	3,301,500	10,466	6,119,546	21,740	12,677,972
Mo.	Minnows	3,500	1,242,500	3,500	1,242,500	1,254	445,170
	Catfish	1,200	564,000	692	325,240	794	373,180
	TOTAL	4,700	1,806,500	4,192	1,567,740	2,048	818,350
Okla.	Minnows	200	61,600	200	61,600	106	37,630
	Catfish	200	94,000	198	93,060	415	195,050
	TOTAL	400	155,600	398	154,660	521	232,680
Tenn.	Minnows	-	-	-	-	90	31,950
	Catfish	-	-	-	-	261	122,670
	TOTAL	-	-	-	-	351	154,620
Tex.	Minnows	650	200,200	650	200,200	480	170,400
	Catfish	2,000	940,000	2,490	1,170,300	2,595	1,219,650
	TOTAL	2,650	1,140,200	3,140	1,370,500	3,075	1,390,050
ALL STATES	Minnows	22,670	7,828,360	23,970	8,252,260	28,587	10,154,385
	Catfish	15,450	8,750,750	24,496	14,185,398	39,921	23,545,858
	TOTAL	38,120	16,579,110	48,466	22,437,658	68,508	33,700,243

1/ Lanier Green, Southeastern Fish Cultural Laboratory, Marion, Ala., states that there are more than 3,000 acres of catfish in Alabama.

2/ Paul Schumacher, Soil Conservation Service, lists 2,992 acres of catfish in Georgia.

THE NEW ENVIRONMENTAL AWARENESS

A number of years ago marine and reservoir fishery research were placed in a Branch of Fish Ecosystem Research. The change in title made no difference in the way the concerned scientists approached their work; it was merely a more precise and logical definition of the field. In short, the new title was more responsive. It was therefore somewhat surprising to find the change caused distress among a few people inside as well as outside the fishery research field. It was this attitude which prompted our attempt several annual reports ago to discuss the ecosystem approach to fishery research. The gist of these remarks was that planning is in terms of communities rather than individuals, and the preferred method is to go to the environment rather than to bring the environment into the laboratory. We went on to point out that the laboratory approach was not by any means scorned and that the behavior, physiology, disease, and biometric aspects all involve extensive bench research activities. Even here, however, the approach is essentially ecological.

If the change in branch title were to be made today, it would hardly cause a ripple in the new atmosphere of environmental awareness where words like ecology, ecosystem, and environment are becoming so commonplace that they are in danger of becoming downright platitudinous!

We are pleased with this new awareness. Some of the scientists in fishery research have spent as much as 40 years of their life working in ecology, and the promise of recognition is sweet. These men know a lot about aquatic environments and some of them bear the scars of numerous conservation battles.

A disturbing note frequently creeps into public exhortatives for support of the new awareness. Too often statements include remarks such as "virtually nothing is known," "little work has been done," or "the problem has received little attention." In reality, a great deal is often known, years of work have been devoted to the subject, and whole institutions have been created to address the problem. In Progress in Sport Fishery Research--1967, the Director of the North Central Reservoir Investigations, after outlining the progress made in defining the relations between reservoir ecology and fish populations, made the remark that, "System ecology is becoming a part of water management in reservoirs." He also pointed out a number of ways in which this knowledge is actually being applied by the U. S. Army Corps of Engineers in the upper Missouri River impoundments. Yet we still hear people who should know better stating categorically that we know nothing or can do nothing about reservoir fishery management. The same general statement can be made about virtually any field of fishery management.

We believe fishery ecologists have a tremendous opportunity to contribute to the well being of the environment in which our society exists. They have the research-based ecological knowledge, the political community recognizes the need for ecological consideration, and there is clear-cut support from an alerted public.

The ecological approach to solving environmental conflicts has never been an easy one. We are hopeful that when the need to apply what may be drastic measures to maintain quality and productivity, the new awareness will be strong enough to square off with the traditional and easily rationalized economic commitments. Will the need to protect a watershed, a swamp, or an estuary for not easily rationalized esthetic, productivity or recreational purposes prevail over the convenience of their uncontrolled use as sources or disposal areas for coolant from a power plant which will permit all the air conditioners in a city to operate? We hope so!

J. Bruce Kimsey, Chief
Branch of Fish Ecosystem Research

ATLANTIC MARINE GAME FISH RESEARCH PROGRAM

Lionel A. Walford, Director

SANDY HOOK MARINE LABORATORY
Highlands, New Jersey

HIGHLIGHTS

In our efforts to explore the biology of game fish, we have been confronted this year more than any previous year with the tremendous influence that man has on the marine environment. At this time many of our studies and observations relate to rapidly changing environmental conditions.

The estuarine zone is one of the most productive, fragile, and yet exploited areas along the Atlantic coast. Estuaries are necessary for survival of the juvenile forms of such coastal migratory game species as striped bass, mackerel, drums, sea bass, flatfishes, and bluefish. Since 1966 we have been systematically collecting and identifying the eggs and larvae of coastal fishes over the continental shelf from Martha's Vineyard, Massachusetts, to Florida. We have determined the times and places of spawning of the economically-important species. That they migrate to the estuarine zone as they develop is shown by the fact that the larvae are progressively larger as we collect closer inshore. We are engaged in detailed studies of estuarine-dependent factors which we began this year with out analysis of juvenile bluefish feeding habits. As the juveniles leave ocean waters to enter estuaries they switch from a diet of plankton to one of small fishes and herrings.

The New York Harbor area, now highly polluted, has become a virtual death trap, at least during the summer months, especially for young fishes of several species. Last summer and early fall the fin rot disease reached epizootic proportions. On the basis of test cultures made from tissues of diseased fish, we have presumptive evidence that marine bacteria are the cause of the infection.

The coastal waters may be destroyed by pollution before we can determine their value. Data collected in the past year as part of a study we are conducting for the U. S. Army Corps of Engineers to determine the effects on marine resources of waste disposal practices in the New York Bight show clearly that 40 years of dumping have done great damage to benthic life. We can now confirm that 10-12 square miles of bottom at the sewage sludge dumping grounds are devoid of life. Part of this condition is due to the low dissolved oxygen content of the water, especially during part of the summer, when the DO is less than 1 ppm, too low to support normal benthic life. A cooperator found that population growth of phytoplankton is inhibited in water from the sewage sludge disposal area. We found up to 250 ppm of lead in bottom deposits and many other toxic agents. The dumping grounds are located in a dynamic current system with bottom water moving over the sludge area towards New York and New Jersey shorelines.

The story out of Puerto Rico, one year after the sinking of the oil tanker, Ocean Eagle, is a happier one. We observed no traces of oil on the lower intertidal zone and invertebrates and fishes there appeared to be again abundant and healthy.

During the year the Coast Guard assumed responsibility for collecting the monthly Atlantic Shelf survey temperature data and we began concentrating our efforts on surveying estuarine areas threatened with thermal loading. We are attempting to obtain baseline temperatures of the areas soon to be exposed to heat loading.

Environmental conditions such as those caused by pollution and physical change often have dramatic effects on the activities of marine animals. But natural factors such as water temperature, length of day, and available food are what determine an organism's daily and seasonal patterns of behavior. We began laboratory studies on summer flounder (fluke), commonly thought to be a relatively inactive benthic species, and found that during the day, except for a period in late summer, the fish swam continually and were active predators. The most surprising discovery was the manner in which fluke would swim vertically towards the surface, then glide gradually towards the bottom, covering long distances with a minimum expenditure of energy.

Most of the economically important sport fishes are migratory species, which move up and down the Atlantic coast in response to seasonal changes in food supply and water temperature. So that knowledgeable management policies can be made, we are making detailed life history studies of several of these species. We have defined populations, migratory routes, growth patterns, and spawning places of bluefish and have just completed our first year of a similar study on the drum family, including croaker, weakfish, and spot.

Awareness of patterns of migration, daily activity habits, definitions of populations, and the effects of environmental conditions has led naturally to attempts at population conservation and enlargement through habitat improvement. Artificial reefs of scrap material have been successful in concentrating game fish all along the coast in areas which were once flat and barren, hence unproductive. In the past year we installed 35,000 tires on two reef sites. We now have 8 experimental reef sites between Florida and Long Island, N. Y. One way we are determining the success of the artificial reefs is by examining the success of the anglers who fish over them. Another way is first-hand observation of the reef community. Dives made over the South Carolina reef site revealed a well-balanced community; sea bass, sheepshead, pinfish, and some invertebrates are dependent on the reef fauna and were observed in active competition for food. Observations made on the wreck Delaware during August revealed conditions of such high turbidity (possibly caused by disposal of solid wastes and dredge spoils) that the ambient light at mid-day was zero. Reef fishes which normally feed during the daytime were wedged in crevices and were so lethargic that they could be touched.

HYDROGRAPHY OF COASTS AND ESTUARIES

Since 1962 an aerial survey team at Sandy Hook recorded biological sightings and collected infrared surface temperatures over Atlantic shelf waters. More than 10,000 observations of fishes, turtles, and marine mammals, in addition to thousands of transect miles of surface temperature, were recorded. These data were subjected to preliminary analyses and sent to any interested individual or institution immediately after each monthly survey.

During the first 6 months of the year we continued monthly aerial surveys to map surface temperatures and marine animal distribution. Since the Coast Guard assumed responsibility in July for routine field aspects of the shelf program, time is now available for complete analysis and publication of these data. Initially we will concentrate our efforts on animal distributions and their relation to surface temperatures.

Biological observations through the October flights were incorporated into our ADP section for correlation analyses of these observations and ambient water temperature.

The use of helicopters for our radiometer surveys proved feasible. Although a helicopter is limited in its range when compared with fixed-wing aircraft, its ability to maneuver and hover offsets this disadvantage (Figure 1). The low altitude maneuverability allows greater precision in navigation and hence better quality of data collected from relatively confined bodies of water, while the ability to hover permits lowering of a thermistor for temperature checks of the remote sensor and for obtaining inflight water samples.

Figure 1.--A jet-powered helicopter used for inshore temperature surveys.

We continued a cooperative study of Block Island Sound with the Naval Underwater Sound Laboratory. A report of the surface heat configuration is nearly completed.

We conducted radiometer surveys of the Connecticut River, Barnegat Bay, and Long Island Sound, all areas either now exposed to thermal additions or expected to be so in the near future. The objective of such surveys is to obtain baseline temperatures before any thermal additions. These will be followed up by surveys to depict variations in the plume of heated water, specifically designed to document the extent and increment of such heating.

A sample of our results on the Connecticut River is shown in Figure 2 as an example of a fluvial survey, having several dams and industrial zones. We prepared a report of a series of surveys for the Technical Committee for Fishery Management of the Connecticut River Basin.

Thomas Azarovitz,
Malcolm Silverman, and
Charles Morrison

ENVIRONMENTAL ANALYSIS

We completed a world distribution map of bluefish. The plot shows a scattered array, with major populations in waters off our eastern seaboard, southern Brazil, Venezuela, South Africa, Australia, and in the Mediterranean and

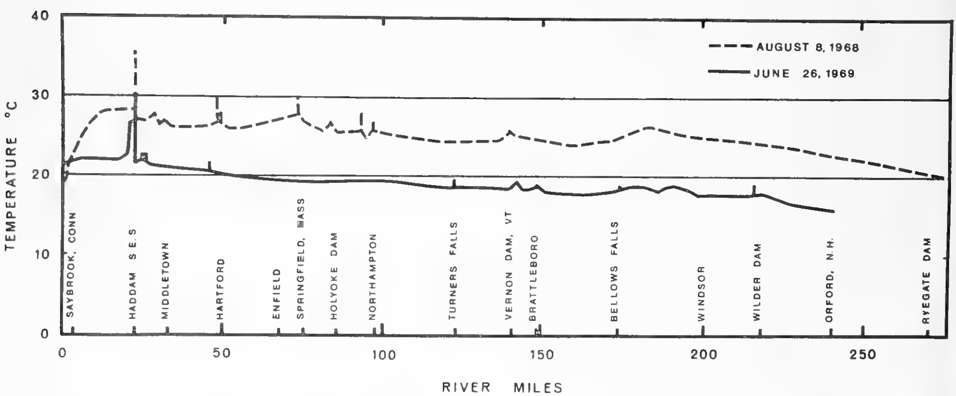


Figure 2.--Surface water temperature of the Connecticut River recorded by radiometer technique on two surveys.

Black Seas; all in temperate zones near the annual range of the 20° C. isotherm. Temperature differences appear to effectively isolate groups of bluefish. One example is the absence of bluefish on the SW coast of South America. This area is apparently isolated from other locations where bluefish live by constant cold water to the south and tropical waters to the north.

We also continued our studies of groundfish distribution in the New York Bight. From samples taken at 24 stations, we gathered basic data on species composition, lengths, and weights for correlation with temperature, salinity, depth, and distance from shore at time of capture.

L. A. Walford,
Robert Wicklund, and
DeWitt Myatt

ESTUARINE DEPENDENCE OF COASTAL FISHES

As the year ended we brought to completion the sorting of the southern series of R/V Dolphin collections. With the processing completed we are now concentrating on analysis and publication of oceanographic and ichthyoplankton data. By the end of 1970 we shall be able to report with assurance on the spawning places and seasons for most economically important estuarine-dependent species that spawn on the continental shelf.

We are now commencing the planning of field work for several subsequent phases of the study. One is a survey within the estuaries of the distribution and ecology of estuarine-dependent juvenile fishes. This would be a large-scale effort requiring a sampling period of 1-1/2 to 2 years. The second is a survey of the Gulf Stream front to discover the mechanism of transport of larvae and juveniles of such species as bluefish from southern spawning places to northern nursery areas. A third phase involves detailed studies of estuarine-dependent factors, as exemplified by the study of bluefish feeding phases reported in the following pages.

John Clark

Plankton sorting

We removed the fish eggs and larvae from plankton collected on southern cruises of the R/V Dolphin and separated the larvae into 26 groups to facilitate final identification. We determined the volume of plankton in the southern samples and prepared these data for presentation in a technical paper. A total of 1,400 samples of plankton collected on our 12 survey cruises from Massachusetts to Florida were sorted. In a check for thoroughness of sort we found an average of 99 percent of the eggs and 96 percent of the larvae had been retrieved. Much of the hydrographic data as well as the distribution and size data for all fish larvae investigated to date are keypunched for computer analysis.

Arthur Kendall

Croakers and mackerels

We examined all Atlantic croaker, spot, and banded drum taken from the survey area between Martha's Vineyard, Massachusetts, and Florida (Table 1). Atlantic croaker larvae occurred from August to April. Spawning time, as indicated by presence of smaller larvae, occurred between August and December. Spot spawned later in the season and appeared in catches during November to May with smaller specimens taken during November and December. Banded drum larvae occurred north of Cape Lookout, N. C. from April to October with a peak in catch during August and on each of the four cruises south of Cape Lookout. A preliminary review of the data indicates a strong positive correlation between distance off-shore and the length of spot larvae; the largest larvae being found nearest to shore.

Eggs and larvae of Atlantic mackerel, Scomber scombrus, from the R/V Dolphin's ichthyoplankton survey were sufficiently numerous to justify preparation of a manuscript describing the egg and yolk-sac larval stages and the distribution of young stages collected during 1966. We completed a first draft summary.

Table 1.--Mean standard lengths of three sciaenids taken in Gulf V plankton net tows by R/V Dolphin, on twelve cruises.

Cruise date	Atlantic croaker Number	Atlantic croaker Mean length (mm)	Spot Number	Spot Mean length (mm)	Banded drum Number	Banded drum Mean length (mm)
Martha's Vineyard, Mass. - Cape Lookout, N. C.						
Dec. 3 - 15, 1965	602	4.2	2094	4.4		
Jan. 25 - Feb. 9, 1966	33	7.5	399	9.2		
Apr. 6 - 22, 1966	2	6.4	24	7.4	1	5.2
May 12 - 24, 1966			3	11.6	7	3.9
June 17 - 29, 1966					3	3.8
Aug. 5 - 26, 1966	263	3.6			90	3.2
Sept. 28 - Oct. 20, 1966	420	3.7			21	3.9
Nov. 9 - Dec. 4, 1966	1039	5.2	54	3.9		
Cape Lookout, N. C. - West Palm Beach, Fla.						
May 7 - 15, 1967			1	11.7	64	3.1
July 22 - Aug. 1, 1967					21	2.6
Oct. 19 - 26, 1967	446	3.6			19	6.5
Jan. 27 - Feb. 4, 1968	110	6.2	2203	6.3	1	5.0
Total number	2915		4778		227	
Weighted mean		4.5		5.7		3.5

Several specimens of the genus Scomber taken south of Cape Lookout appeared morphologically distinct from Atlantic mackerel and were tentatively identified as chub mackerel (*S. japonicus*). Several criteria including vertebral counts, body proportions, and pigmentation were used in separating the two types. Figure 3 depicts one separating feature, the relatively shorter preanal length of Atlantic mackerel. We continued to compile reference material as an aid to separating and identifying other scombroids in the collections.

Peter Berrien

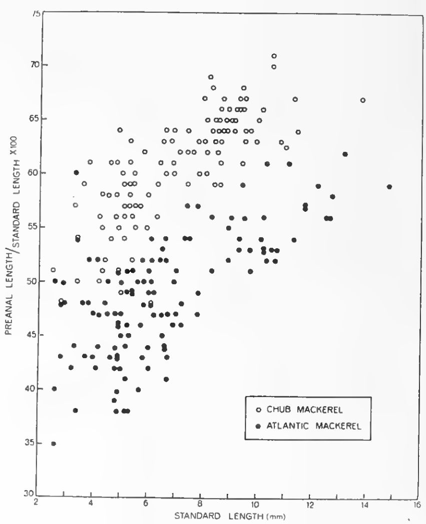


Figure 3.--Data point array showing body proportion differences between Atlantic and chub mackerel.

Cods

We completed sorting all larval gadids collected during the northern series of cruises (1966) which include larvae of Atlantic cod, pollock, haddock, cusk, fourbeard rockling, silver hake, and hakes of the genus Urophycis.

We placed initial emphasis on the silver hake, Merluccius bilinearis, collecting a total of 11,316 larvae throughout the year. We isolated, counted, and measured all silver hake larvae and plotted their geographic relative abundance. We are preparing these data for publication. Collections within the 1966 sampling area (Figure 4) indicate a prolonged spawning season, extending from May into December with a peak from August to October. Geographic distribution of larvae was similar during all months the species was taken. We made the largest catches on transects off Martha's Vineyard, Massa., and Montauk Pt., N. Y. We found larvae increasingly restricted to the offshore ends of more southerly transects. Generally, we captured smaller larvae north, inshore and near the surface and larger larvae south, offshore and deeper (Table 2).

We began examining a series of Merluccius larvae, presumed to be M. albidus. The postlarva of this species is at present undescribed.

Michael P. Fahay

Sea Bass

Black sea bass larvae, recognized from our plankton samples north of Cape Lookout, have not been described in the literature. Our series includes 135 larvae ranging from 2.1 to 11.8 mm long. These larvae occurred from June through October over the middle of the continental shelf from New Jersey to North Carolina (Figure 4). We identified the larvae by meristic characters and a characteristic series of ventral pigment spots (Figure 5). The absence of larger specimens in plankton and midwater trawl samples and their reported occurrence inshore as juveniles indicate an estuarine dependence for the species.

Arthur Kendall

Flatfishes

Our efforts centered on species identification, analysis of findings and preparation of manuscripts from data collected during the two-part coastal ichthyoplankton survey of 1965-66 and 1967-68. Figure 6 depicts the density

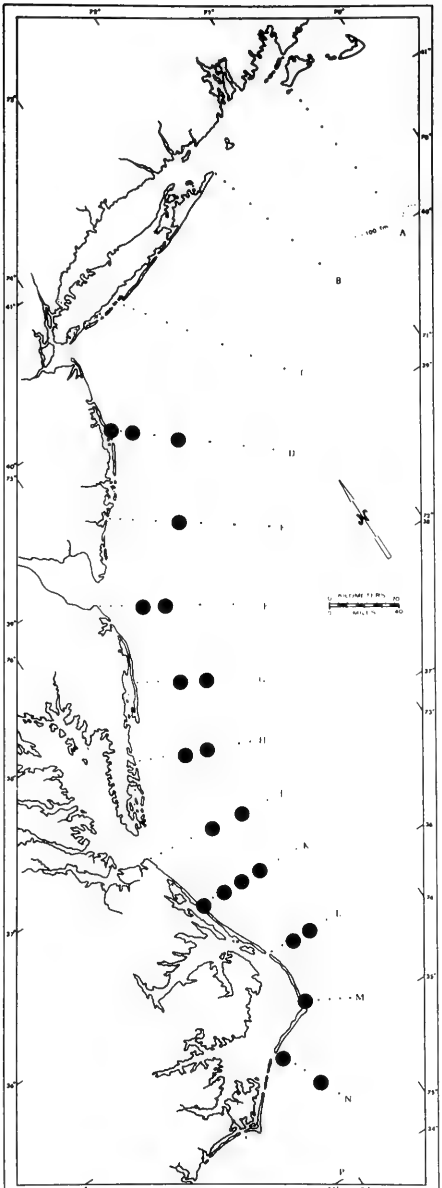


Figure 4.--Capture locations of black sea bass larvae from R. V. Dolphin survey cruises. Regular sampling stations indicated by the array of smaller dots.

Table 2.--Mean lengths of silver hake larvae collected during an August cruise (R/V Dolphin, D-66-10).

			Distance offshore (nautical miles)								Weighted	
			5	10	15	25	35	50	65	80	mean	
Transect	Depth	State	mm (Notochord length)								length	N
A	S	Mass	3.2	2.8	4.0	3.1	5.0	4.0	3.6	ns	3.8	598
	D				5.8	5.1	5.3	5.8	4.2	ns	5.6	658
B	S	NY	2.8	3.8	2.0	3.9	4.1	5.4	6.6	ns	5.4	4.3
	D			3.6	3.3	5.5	6.2	6.8	8.5	ns	7.8	1037
C	S	NY	2.1		3.2	3.6		3.2		4.9	3.5	54
	D					4.7	3.5		5.3	5.4	5.3	98
D	S	NJ					3.8	5.8	6.0	3.6	5.6	33
	D						3.2		12.2	10.2	10.3	71
E	S	NJ						5.3	3.3		4.0	3
	D							6.0	5.5		5.7	8
F	S	Del						6.0	7.5	ns	7.3	8
	D							7.0		ns	7.0	2
G	S	Md					3.6			ns	3.6	1
	D									ns		
H	S	Va					14.8			ns	14.8	1
	D						14.5		15.1	ns	15.0	3
J	S	Va							5.7	ns	5.7	1
	D									ns		
Weighted mean length			3.0	3.2	4.2	3.6	5.1	5.6	7.9	7.4	6.1	
N			18	67	82	357	472	853	987	153		2089

Shallow net (S) samples 0-15 m; deep net (D), 18-33 m. ns, not sampled.

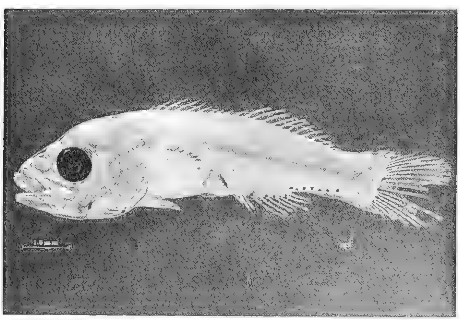


Figure 5.--Black sea bass larva, 7.9 mm long, from R. V. Dolphin ichthyoplankton survey cruises.

patterns of summer flounder eggs and larvae collected during 1965-66. Eggs were present during three cruises. We collected summer slounder larvae regularly during October, November, and December, and occasionally dur- January, March, and April, 1966. Larval concentrations centered off New York, New Jersey, and Delaware in October, 1966, and off New Jersey in December, 1965 and 1966. With few exceptions larvae occurred only between Cape Hatteras and Cape Lookout during the January, March, and April cruises.

We made three unsuccessful attempts to find postlarval and juvenile summer flounder in areas other than the North Carolina estuaries, where

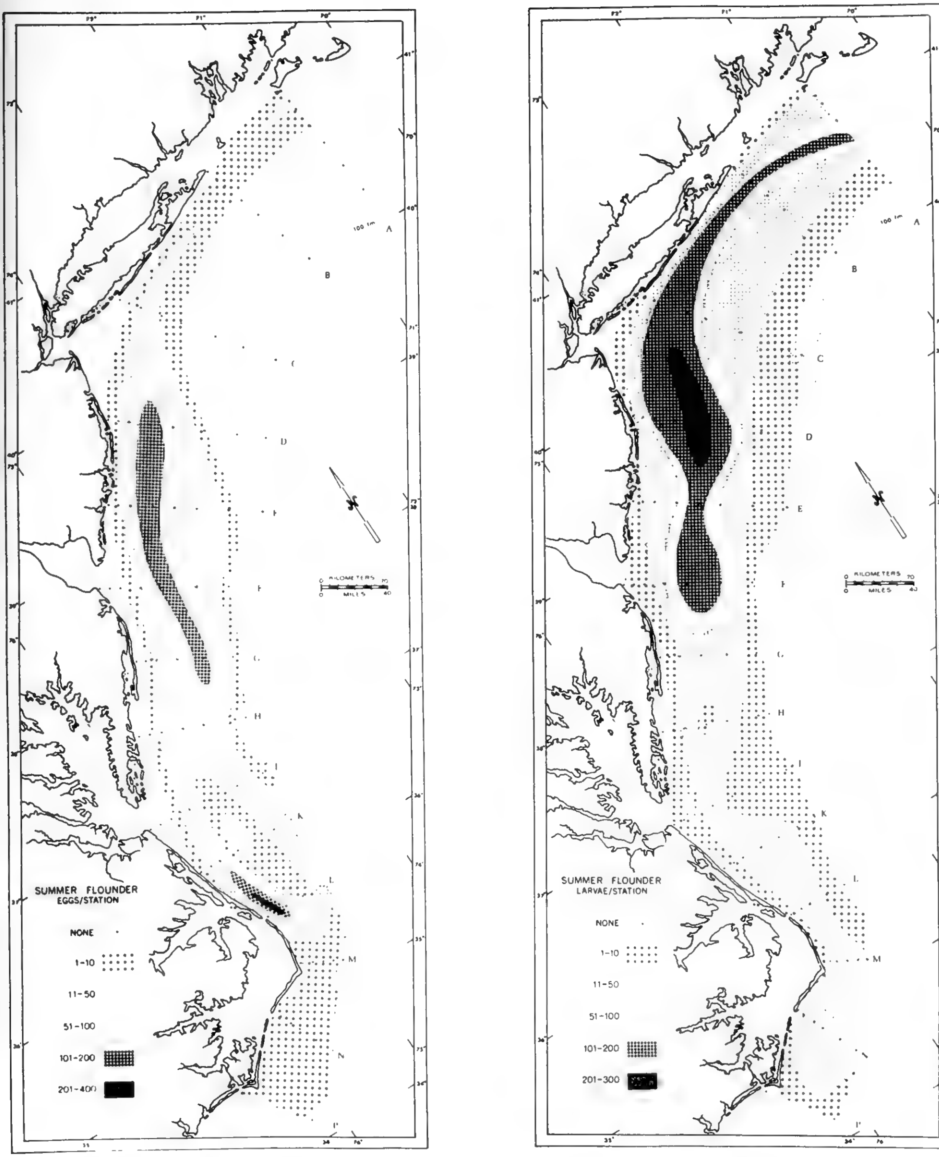


Figure 6.--Distribution and relative abundance of summer flounder eggs (left) and larvae (right), from R. V. Dolphin ichthyoplankton survey cruises, 1965-66.

they occur regularly. During February and May we sampled with a 3/4-scale Yankee trawl in depths varying from 13 to 91 m at 60 stations along the continental shelf between Cape Hatteras, N. C., and Sandy Hook, N. J., and in June, seined at 32 shore stations in estuaries of eastern Virginia, Maryland, and Delaware. We followed up several reports of small summer flounder taken in local estuaries but in every instance caught only juvenile winter flounder.

W. G. Smith

Estuarine occurrence of juveniles

We continued to receive and prepare contributions to a review of data on the eggs, larvae, and juveniles of estuarine fish of the Atlantic coast. The publication is a product of cooperative effort between the Atlantic States Marine Fisheries Commission and both Bureaus of the service, who jointly sponsored a workshop meeting in 1968. We corrected and revised one particular section which summarizes geographically, by species, the known literature on distribution of juveniles.

A. L. Pacheco

Bluefish origins

In June, we sampled for occurrence of juveniles between Cape Cod, Mass., and Barnegat, N. J., as far as 280 miles offshore into the Gulf Stream and took bluefish only near shore. The capture of some juveniles of mullet and goatfish--typically shallow-water forms--in and near the Gulf Stream, indicated a transport mechanism from southern waters. Apparently a body of juvenile bluefish had moved shoreward before our cruise, probably because of an early warming of coastal water. We also sampled for juvenile bluefish around Sandy Hook from April through October to determine their seasonal occurrence and habitat preference. Of particular interest was the occurrence of several 50 mm bluefish in October night-lighting collections, suggesting an earlier mid-summer spawning.

Arthur Kendall

Food habits of juvenile bluefish

We carried out a preliminary study of the feeding habits of juvenile bluefish to relate the movement of juveniles into estuaries with changes in feeding habits. The result demonstrates a shift in feeding from plankton to small fish and shrimp when juveniles reach a length of about 75 mm (3 in).

We examined stomach contents of nearly 300 juvenile bluefish, taken from the ocean, bay, and river habitats surrounding Sandy Hook, N. J. Food occurred in 66 percent of the stomachs; 26 percent were empty, and 8 percent contained slurry, sand, or plant debris. Of the fish which had eaten, 48 percent contained fish and 55 percent contained invertebrates. Of the major food items, silversides ranked first in frequency of occurrence (33 percent), and accounted for 75 percent of the aggregate food volume. Copepods ranked high in frequency of occurrence (28 percent), but made up only 1 percent of the total food volume. One copepod species occurred in 18 percent of the stomachs containing food. Shrimp, especially the estuarine species Crangon septemspinosa, ranked next in total food volume (12 percent).

Fish under 70 mm contained mainly copepods, fish eggs and crab larvae, while those over 70 mm contained mainly shrimp and fish. This change in diet with size (Figure 7) also coincides with a change from ocean to estuarine habitat.

Susan Smith and Ann Gall

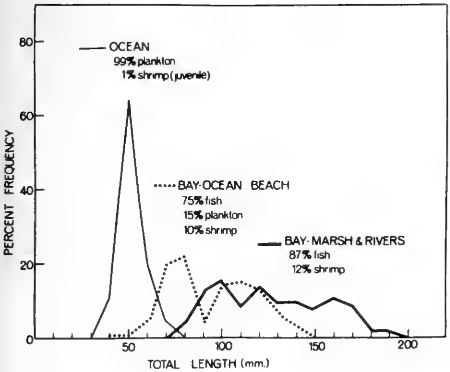


Figure 7.--Variations in young bluefish stomach content in relation to fish sizes and habitat.

Figure 8.--Healthy (upper) and fin rot infected (lower) bluefish.

SPECIAL MICROBIOLOGICAL PROBLEMS IN ESTUARINE AND COASTAL AREAS

Study of two important and recurrent local problems, a fin rot disease and red tide, continued during the year. Work consisted largely of monitoring the occurrences. Study of both problems will be expanded in 1970 to include tests to determine their causative mechanisms.

Fish disease study

The fin rot disease (Figure 8) again reached epizootic proportions in fishes in the Raritan Bay, Lower Bay, and Sandy Hook Bay area. Table 3 provides the incidences of the disease throughout most of the year in the four principal species affected.

In November, following an inshore movement of white hake, we observed the disease in this species for the first time. We had not observed the disease in smaller white hake, summer residents in the Bay area. Subsequently, trawling in Raritan Bay, Lower Bay, and Sandy Hook Bay yielded catches of white hake with incidences to 60 percent whereas catches made at several ocean stations in a line starting 12 miles east of Sandy Hook to a point 5 miles south were almost free of diseased fish. Of over 700 white hake collected in ocean stations, we obtained only a few diseased specimens in the vicinity of ambrose Light. South of Ambrose none showed the disease.

We completed generic characterization of 100 bacteria cultures isolated from the necrotic fins and internal tissues of diseased fish sampled during the 1968 epizootic. Most of these (83) belonged to three closely related genera: Aeromonas, Vibrio, and Pseudomonas. We have presumptive evidence that the bacteria are marine. Tests of the effects of NaCl on growth of these cultures revealed that 98 either did not grow at all in the absence of salt or had greatly increased growth when salt was added. We initiated a year-round program of blood testing, including hematocrit, hemoglobin, red blood cell counts, and serum protein determinations on fresh-caught winter flounder to measure the relation of blood quality with disease incidence.

Red tide

Red tide was not a serious problem in the Sandy Hook area in 1969. On July 8, a bloom developed in a square mile area of the

Table 3.--Incidence of fin rot disease in principal species affected during 1969 in the Raritan Bay, Lower Bay, Sandy Hook Bay area.

	March to May	June	July and August	September too November
	Percent diseased			
Winter flounder	1	5	20	< 1
Summer flounder	-[1/]	25	28	-
Bluefish	-	2	13	6
Weakfish > 20 cm standard length	-	-	16	10
Weakfish > 10-20 cm standard length	-	-	66	100

[1/] Dash symbol signifies fish either not in the area (migratory species) or absent from collections.

Shrewsbury River, consisting mostly of Prorocentrum micans (9,000 cells per ml) and Massartia rotundata (80,000 cells per ml). The next day, in another part of the river, an Olisthodiscus luteus bloom reached 25,000 cells per ml. We observed the first and only major dinoflagellate outbreak in the ocean from July 10 to 13. The bloom extended 15 km southward from the tip of Sandy Hook to Sea Bright, N. J., along the shore and out to 100 m. We examined samples of bloom water and found four species of phytoflagellates present in about equal numbers. These included the species mentioned above and Euglena sp. Total cell counts reached 60,000 cells per ml. For comparison the more severe blooms of 1968 contained 600,000 to 800,000 cells per ml. We believe the combined effects of heavy rainfall, colder water, and prevailing southerly and westerly winds prevented dinoflagellate blooms developing as much as in the previous summer. From August through November the dominant phytoplankton in the area was a diatom, Skeletonema costatum.

John Mahoney

OFFSHORE WASTE DISPOSAL

Dredge spoils and sewage sludge have been dumped at designated disposal areas in the New York Bight for 40 years. Our research in the past two years has revealed that these activities have had a major effect on the ecology of marine organisms which habituate the disposal areas and surrounding environments. In the final phase of the study we plan to determine: 1) the rates of accumulation of wastes and spread of impoverished bottom areas, 2) uptake of toxic pollutants in marine food chains, 3) possible effects of spoil and sludge wastes on coastal waters and beaches along Long Island and New Jersey, 4) effects of pollutants on a variety of organisms held under controlled laboratory conditions, and 5) relationship of zooplankton, phytoplankton, and pelagic organisms to the waste disposal areas. We also plan to survey and census additional bottom areas which might serve as more appropriate temporary dumping sites as well as conduct experimental dumping to determine the immediate and long-term effects of dumping in a previously uncontaminated environment. The project ends in Fall 1970 unless further funding is provided by the support agency, the Army Corps of Engineers.

Effects of offshore waste disposal practices on marine resources of New York Bight

Our analyses of quantitative bottom grab samples and physical data from 221 benthic stations confirmed our initial estimates that 10-12 square miles of bottom at the sewage sludge dumping grounds are devoid of life. An area of about 5-7 square miles in and around the dredge spoil disposal grounds proved to be even more heavily damaged.

The bottom sediments at the sewage sludge and dredge spoil disposal areas are characterized by their high content of organic matter and heavy metals (Figure 9). The distribution of these materials confirms the patterns of water movement indicated in the hydrographic study. We also found sediments collected from the dredge spoil disposal area to contain over 1 percent petrochemicals. Many of the dredge spoils originate in harbors polluted with petroleum products.

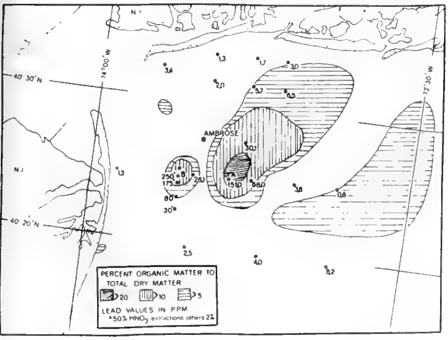


Figure 9.--Distribution of organic matter and lead in sediments of the northwest section of the New York Bight. High levels are associated with the disposal areas for sewage sludge (A) and dredge spoil (B). (cf. also Figure 11.)

We started a series of experiments to determine whether the heavy metals are taken up by benthic organisms and, if so, how they are moved through the food chain.

We observed that water overlying the bottom sediments at the dredge spoil and sewage sludge dump areas contains much less dissolved oxygen (DO) than water overlying unpolluted sediments at similar depths. Dissolved oxygen in water over the sludge grounds falls below 1 ppm for several weeks during summer months, far less than that required to support normal benthic life. The low DO undoubtedly results from the high biological and chemical oxygen demands associated with various organic wastes dumped at the two disposal sites. The movement of this extensive body of water with low DO may account for the mass mortality of several fish and invertebrate species in September, 1968, off the New Jersey coast.

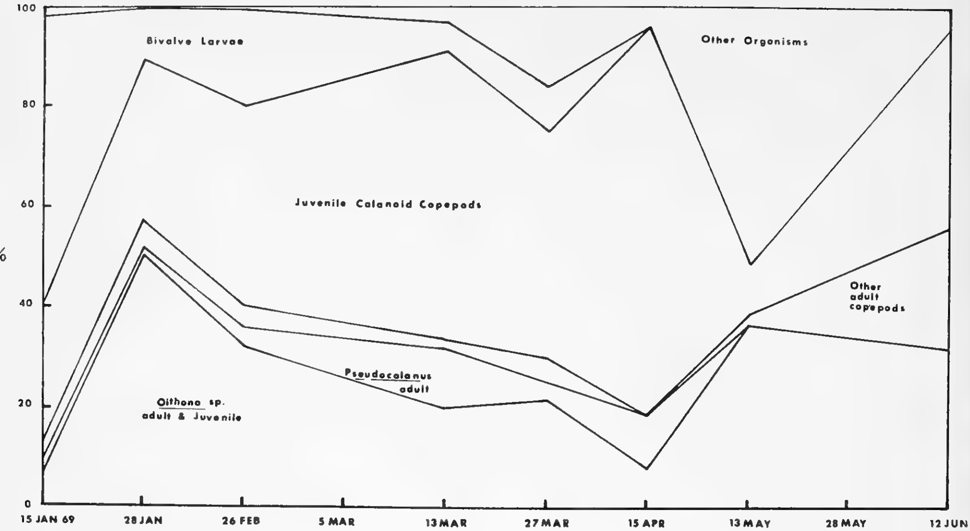
Our analysis of benthic macrofauna indicates that the distribution of benthic invertebrates is affected by the presence of sewage sludge or dredge spoils. Benthic communities surrounding the areas devoid of life are of low diversity (few species), generally dominated by a burrowing sea anemone, Cerianthus, and a rubber worm, Cerebratulus. We analyzed the distribution of 23 species of amphipods collected with soft sediments and found only one species (Unciola irrorata) in the disposal areas. Our observations of the meiofauna (forms ranging in size from 0.25-1.00 mm) indicate that nematodes, one of the few groups generally regarded as resistant to organic pollution, are not found in the fludge and dredge spoil disposal areas.

Laboratory experiments to determine lethal and sublethal effects of sewage sludge and dredge spoils on benthic organisms showed exposure to sludge resulted in severe pathological anomalies and death in crabs and lobsters. In preference and avoidance experiments we found most organisms avoided contact with sludge if a more suitable substratum was available.

Richard Barber, Woods Hole Oceanographic Institution, cooperating with us in studying the effects of sewage sludge on phytoplankton populations, found that water collected from the sludge disposal area inhibits cell division and, thus, population growth. He did not isolate a causative factor. Water from other stations in the Bight did not inhibit growth.

Personnel of the PHS-FDA Laboratory at Davisville, R. I. found sediments from the sludge disposal area contaminated with coliform bacteria. Their counts in the sludge area were higher (160,000 MPN) than in uncontaminated sediments 5-6 miles from the center of the disposal area.

We studied effects of waste disposal on the biology and distribution of the surf clam, Spisula solidissima, and the rock crab, Cancer irroratus. The former is an important commercial species found in the vacinity of both the



STATION 76-77 - SURFACE

Figure 10.--Percent composition of zooplankton groups in surface waters at one sampling station from January to June.

dredge spoil and sludge disposal areas. We are following the development of a particularly heavy set of clams which occurred in 1968. The rock crab, a dominant food species utilized by finfishes of the New York Bight, is also a potential market item.

Zooplankton samples are used to determine the distribution and abundance of organisms (Figure 10) in relation to sewage sludge and industrial acid wastes.

Chemical analyses of sea water showed the water overlying the sewage sludge disposal area to have a high organic and inorganic phosphorous content in comparison with the surrounding waters.

Preliminary data from trawling surveys indicate bottom-dwelling fishes are present in the disposal area during late spring and early summer. We did not find fishes in the late summer when DO levels were less than 1 ppm. Analyses of stomach content indicated fishes collected in the disposal area were feeding on pelagic and planktonic organisms.

Jack B. Pearce, Charles Gibson, and Andrew Draxler

Temperature and current study

Our hydrographic study of the New York Bight area shows the present sludge dumping ground to be within an apparent dynamic current system. Inshore and longshore movements of bottom water are its most obvious and persistent features.

Our source of information comes from an array of 26 sampling stations. At each station we measured water temperature and salinity at 4 m depth intervals, the amount of dissolved oxygen at the bottom and at selected sites, and we released bottom and surface drifters. At four stations we located instruments to measure continuously the current velocity, direction, and temperature at a depth of 15 m and at the bottom.

Preliminary examination of the hydrographic data and the recovery locations of 1,200 drifters gave us an indication of the seasonal variations in current which will be useful in predicting movements of sewage sludge from the dumping site.

We also found indications of a movement of bottom water northward up the Hudson Channel, trending to the northeast at its head (Figure 11). From an analysis of water column sections this showed up as a relatively warm, high-salinity dome of water over the channel during winter and a tongue of relatively cool water in summer.

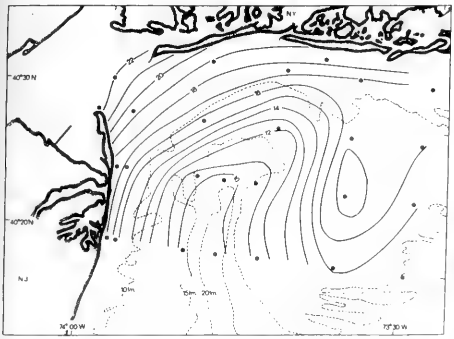


Figure 11.--Distribution of bottom water temperature in the disposal study area of the New York Bight.

We have evidence, from distribution of bottom temperature data, of a southerly flowing countercurrent further offshore during the summer.

The returns of current drifters support the conclusions from station data. The recovery pattern of bottom drifters showed a northerly and inshore pattern, whereas the surface drifters, entrained in the longshore current, were returned from southerly points.

Robert Wicklund and David Hansen

BLUEFISH BIOLOGY

During 1969 we continued analyses of data collected since 1963 to define various aspects of the life history and population structure of the bluefish. From studies on age and growth, larval occurrence, morphology, and migrations, we have now defined two major populations of bluefish along the Atlantic coast. We are continuing to correlate data from the various studies and plan completion of our bluefish studies by the end of 1970.

Natural history

We continued to study differences in body proportions to test our hypothesis of coastal contingents. We measured and photographed samples of bluefish from Pamlico Sound, N. C. and Sandy Hook Bay, N. J. A discriminant function analysis of 13 ratio measurements indicated 91 percent of the individuals could have originated in the sampling locality. We have collected additional data for use in improving the analysis (Figure 12).

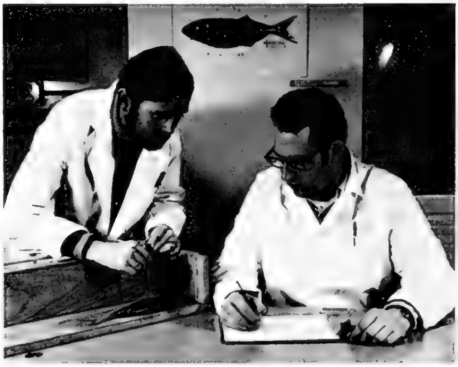


Figure 12.--From a series of systematic measurements, biologists are learning of fish population differences.

We continued to receive tag returns from the 1,106 tagged bluefish released during the summer and early fall of 1967. These tags bore a request for return of a scale sample with other recapture information. Of 64 tags returned 62 percent included scale samples.

All scales returned this year had two additional annuli, further confirming our interpretation of scale characters as indicators of age. One fish, at liberty for 755 days, grew nearly 40 cm, a rate in agreement with our growth estimate.

We collected more bluefish in the 8 to 15 lb (4 to 8-year-old) category to supplement the length-weight and age-weight data we have on hand.

Between May 13 and 23, we made a R/V Dolphin cruise to fish water over the Hudson and Wilmington Canyons by trolling and gill netting for evidence of bluefish migrating toward the coast from offshore wintering grounds, but caught no fish. At most stations water temperature was below 17 to 22 C.

L. A. Walford,
S. J. Wilk, and
M. J. Silverman

Migrations

We are still reviewing returns from the 15,699 bluefish tagged from 1963 through 1967. Nine tag returns came in during the year from fish caught in New York-New Jersey waters. Four of the fish had been at liberty since 1965, the longest time out to date of tagged bluefish.

We have had tags returned from 98 bluefish which had been at large for one year or more. Of these, 80 fish were caught after one year, 10 after two, 4 after three, and 4 after four years out. Most of these longer-term recaptures came from either the area of release or an adjacent area (Table 4).

Using ADP techniques, we completed summaries of all fish released and recaptured, grouped by data and area, fish size, tag type, and fishing gear of capture and recapture. We began processing these data to detect differences in movements, rate of movement, and to estimate apparent mortality indices.

David G. Deuel

BEHAVIOR OF GAME FISHES

Behavior of summer flounder

After studying patterns of behavior for more than four years in the bluefish, a pelagic species, we began studies on a semi-benthic species, summer flounder (fluke). Our aim was to observe daily and seasonal patterns of activity, feeding behavior, and response to temperature. We added a sand bottom to the aquarium and lowered daytime light intensity to simulate the type of habitat in which the fish normally reside.

In May we introduced six fluke ranging from 50-70 cm into the aquarium. Our preliminary observations showed that there were several basic patterns of behavior. At times the fluke would lie flat on the sand surface, eyes retracted, apparently unresponsive to movements of prey or other fluke. At other times, when lying on the sand, their eyestalks were extended, and they were seemingly more responsive to movement around them. Frequently the fish partially raised themselves off the bottom, supported by their dorsal and anal fins, with their heads up and each eye moving independently. In this posture they showed a high degree of responsiveness. Fish, partially or fully buried, were characteristically unresponsive. To bury, the fluke would vigorously beat both head and tail against the bottom, throwing sand up with their fins until partially or completely covered.

The fish swam at every level in the tank from bottom to surface. One of our most interesting observations regarding swimming was the ability of the fish to glide considerable distances with a minimal expenditure of energy. A fluke would swim vertically toward the surface, position its body horizontally and then, by changing his head position, glide to the bottom. The fish would use its body position to control its forward speed and descent, in conjunction with caudal and median fins. Gliding is apparently an important example of an adaption for a migratory fish which expends high levels of energy for swimming.

Table 4.--Summary data of bluefish tagging, 1963-1967.

Recapture area	Release area: Mass. R. I.	Conn. and N. Y.	N. J.	Del. and Md.	Va.	N. C.	S. C.	Fla. (East)	Total
	Numbers recaptured between 1-4 years at liberty								
Mass., R. I.		1	1						2
Conn. and N. Y.	1	43	8						52
N. J.		19	12		1			1	33
Del. and Md.		1							1
Va.					1				1
N. C.			1			1			2
S. C.									
Fla. (East)								6	6
Fla. (West)					1				1
Total at large longer than 1 yr.	1	64	22		3	1		7	98
Total at large longer than 2 yrs.	1	15	2						18
	Numbers of fish								
Total returned	2	151	322	0	76	79	5	470	1,105*
Total tagged	30	3,318	5,694	20	968	1,110	79	4,480	15,699

*Does not include 9 illegible tag returns.

Seasonal patterns of behavior were reflected in changes in daily activity. In June the fish swam continually night and day, taking only occasional rests of 2 to 4 minutes, lying on the sand. After 5 weeks the fish almost completely ceased swimming at night. Then from early to late fall, the fluke remained motionless on the sand for long periods, finally resuming their day-active pattern which continued through the winter. This change may be related to their activity during their normal migratory period.

We began studies of feeding habits and fed the fluke grass shrimp once a month. We found three basic methods of feeding:

(1) fluke, lying on the bottom, would visually fix on a shrimp 12-25 cm away, and while raised on its dorsal and anal fins, inch forward slowly stalking the prey and then, arching its back, strike and ingest the shrimp. Occasionally while stalking, a fluke would trap a shrimp in a sand depression, then proceed to strike and ingest. After feeding, the fluke settled on the sand or swam slowly around the tank bottom before resuming feeding.

(2) fluke would swim vertically towards the surface usually within several inches of the perpendicular side of the tank, sight a shrimp, grasp it directly off the tank wall, then turn and glide or swim around the tank until sighting another shrimp.

(3) fluke, while swimming at intermediate levels in the water column would visually fix on a shrimp, swim towards it, pause for 1-2 seconds, and with an intense caudal downbeat, lunge forward and ingest the prey.

Bori Olla, Anne Studholme,
Dale Martin, Carol Samet, and
Kenneth Hirsch

Schooling behavior

We began preliminary observations on the schooling behavior of mullet as part of our continuing studies to define normal patterns of behavior in marine fishes. Since there is a great deal of evidence that visual cues act as primary stimuli for drawing schooling fish together, it was our intention to examine whether mullet were attracted to each other by visual cues alone.

We separated two adjacent tanks by an opaque partition and placed a single fish in one tank and a group of two to three fish in the other. By removing the partition we were able to examine the responsiveness of the isolate to its species-mates. We found that when the isolate saw the group it responded positively by swimming immediately to the exposed wall where it continued to bump vigorously for the duration of the 15 minute test. This indicated to us that the isolate was highly motivated to join its species-mates, and that its behavior was stimulated solely by visual cues. Each of the isolated mullets maintained a maximum response to the group fish for eight 15-minute exposures over a 2-day period. After this time we found that the response of the isolate to the group diminished significantly.

To define the specific visual cues to which the mullet respond, we are currently exposing isolated fish to stationary and moving models of different shapes, and models with eyespots in different locations. By using these techniques we also plan to study the animals' responses to the more subtle effects of such stresses as temperature changes which are not necessarily detected by other experimental methods.

Bori Olla, Kenneth Hirsch, and
Carol Samet

Retinal changes in winter flounder

Preliminary studies on the winter flounder eye indicated that it adapts to light and dark by a series of positional changes in retinal elements. Changes in the position of the cones and pigment epithelium correspond to the diurnal activity of the animal in the field.

To examine the influence of internal factors on these changes, we held flounder under constant dark conditions for 72 hours. For the first 24 hours we found the cones changed positions at the time corresponding to real sun time. The pigment epithelium, in contrast to what we observed under natural conditions, remained stationary. For the next 48 hours, there was no change in either the position of the cones or pigment epithelium. The influence of an internal mechanism was slight.

Bori Olla and Dale Martin

Underwater observations on fish behavior

We made SCUBA dives to observe the endemic fish population near Fire Island, N. Y. particularly noting fish response to current. Species included tautog, cunner, young weakfish, puffers, sea robins, and kingfish. We consistently found the young of most species avoided strong tidal currents and maintained position by seeking shelter behind various bottom obstructions. The strong currents also inhibited feeding and spawning activity of several adult species. During slack water periods, feeding, spawning, and juvenile activities increased.

Bori Olla, Robert Wicklund, and
David Hansen

NATURAL HISTORY OF DRUMS

We began our study of the sciaenid species in late 1968, to determine distribution, migratory patterns, age and growth, and ascertain through morphometrics racial composition of present stocks. In the forthcoming year we plan to extend our present sampling area (Cape Cod to South Carolina) south to Florida.

During cruises from Charleston, S. C., to Shinnecock Inlet, N. Y., we tagged and released 3,357 Atlantic croaker and 507 weakfish. We received only two tag returns; one from a weakfish tagged and recaptured near Cape May, N. J., and one from a croaker tagged near Morehead City, N. C., and recaptured near Myrtle Beach, S. C. Our catch data indicated the following occurrences: concentrations of yound-of-the-year weakfish from Ocean City, Md., to Sandy Hook Bay, N. J., with large weakfish regularly occurring in the New York Bight area; large aggregations of spot between South Carolina and southern New Jersey showing greatest abundance in the Capt Lookout, N. C. and Ocean City, Md., areas; young-of-the-year and yearling croaker from South Carolina to Chesapeake Bay but concentrated in the Cape Lookout area. We encountered no concentrations of older croaker in any of our sampling areas.

We began an analysis of sciaenid scales to determine age composition and growth characteristics of the six species under study. From a food habit analysis of 125 weakfish sampled from Sandy Hook Bay, N. J., we learned that grass shrimp was the dominant food.

Stuart Wilk and Myron Silverman

INVENTORY AND ATLAS OF SPORT FISHING FACILITIES

"Anglers Atlas of the United States Atlantic Coast" is the title of our forthcoming, four-color guide book. We completed the 35 maps which detail over 75,000 square miles of inshore waters and 28,000 miles of coastal land. We have included the most recent available information on location of fishing reefs, fishing access points, and boat and angler facilities from Passamaquoddy Bay, Me., to Plantation Key, Fla.

We rechecked all cartographic details, typeset, and completed the 115 illustrations of game fish. We readied the typeset tabular and text material to accompany the illustrations and completed draft of the glossary text. The project will be completed with submission of the manuscript to the printer this spring.

Bruce Freeman

ARTIFICIAL REEF DEVELOPMENT AND MANAGEMENT

During the first four years of our artificial reef program we found answers for many of the questions we posed at the inception of this study. Some of the information we can now provide includes: 1) the cost and methods of building reefs with several different materials, 2) life expectancy of car body reefs, 3) techniques to use in building effective tire reefs, 4) which substrate appears to be most effective for colonization by epibenthic organisms, and 5) feeding habits of various fish on artificial habitats.

There are still many questions we are trying to answer. One of the problems that has confronted us throughout our study is highly restricted visibility on our artificial reefs in the New York Bight because of turbid water conditions. We had hoped to obtain quantitative data on fishes and study their behavior on artificial habitats through the use of SCUBA. With poor visibility, however, this has proved impractical.

With the addition of two reefs, one off Sea Girt, N. J., and the other off the coast of southern Georgia (Figure 13), we now have 8 experimental reefs under study. We gave technical assistance to groups creating 8 more reefs along the east coast, two off the coast of New York, one in Chesapeake Bay, three off the coast of South Carolina, and one each in Georgia and Florida. We completed a preconstruction survey and site selection off Chincoteague, Va., in a cooperative experimental reef effort between the Chincoteague National Wildlife Refuge and the Sandy Hook Marine Laboratory.

Our cooperative study with the Environmental Control Administration's Bureau of Solid Waste Management investigating the use of scrap tires as artificial reefs was highlighted by the installation of 35,000 tires on two reef sites in the New Jersey-New York area. We tested different techniques of incorporating scrap tires as reef-building material in configurations that provided necessary relief, ease of handling, and low cost. These are necessary criteria if the material is to be practical for use by sport fishing groups and conservation agencies. After selecting a combination of rod units (Figure 14) and single tire units (Figure 15), we deposited 30,000 tires between June and October on the Atlantic Beach artificial reef off southern Long Island. We then deposited 5,000 tires in November on our new experimental reef site off Sea Grit, N. J.

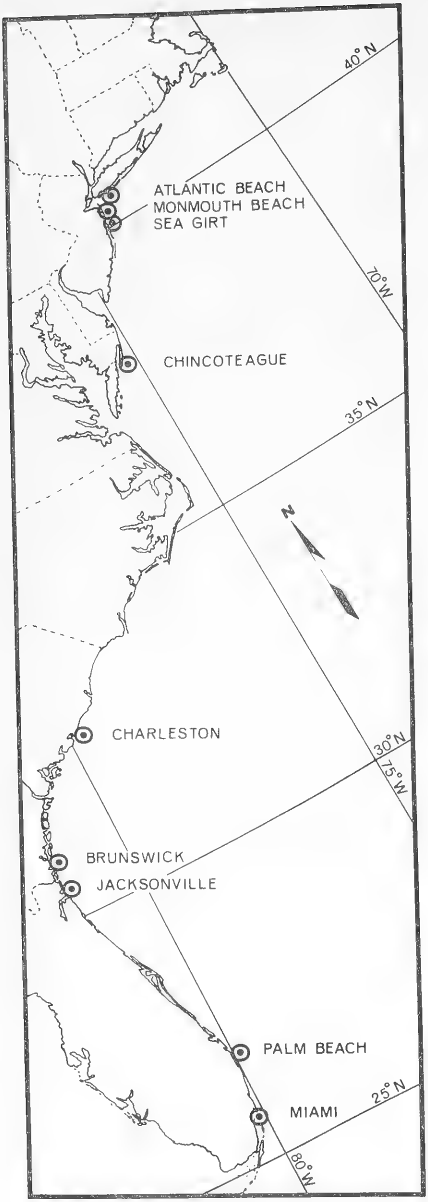


Figure 13.--Location of experimental reef sites under study.

Figure 14.--Barge loading 7-tire units. The units have concrete ballast and are held together with tie-rods.

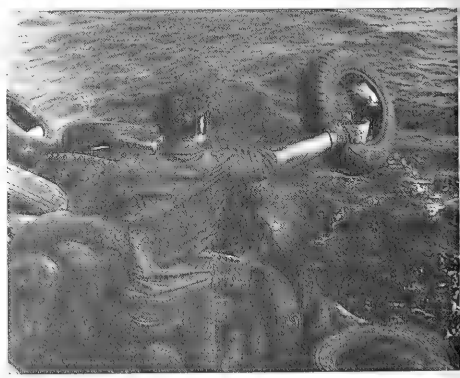


Figure 15.--Individual weighted tires added to an artificial reef increase its functional profile.

Our inspection dives on the Jacksonville and Palm Beach, Fla., reefs revealed numerous game fishes of many species and a thick growth of encrusting organisms on the materials at both reefs (Figure 16). The car bodies on the two-year-old Jacksonville reef showed appreciable deterioration. The car frames remained intact and supported a considerable growth of invertebrates but the thin metal of the roof and sides of many cars had disappeared.

To compare the biomass of encrusting organisms on artificial reefs with populations on natural bottom around the reef, we resumed and refined the tabulation of data collected on a benthic survey off southeastern Long Island from February 1966 to January 1967. Two polychaetes were tentatively identified as new to this area. We found three types of invertebrate distribution present in this area, two specific and one ubiquitous.

Richard Stone and Chester Buchanan

Creel survey technique

We developed and tested several creel survey methods for estimating fishing pressure, catch per angler hour, and anglers' total harvest around artificial reefs. We defined the angling population in our study area as all sport fishermen fishing beyond the surf zone between Manasquan Inlet, N. J., and Jones Inlet, N. Y. To sample this population, we divided the anglers into two groups: 1) party and charter boat anglers and 2) private boat anglers.

In our first attempts to gather information from party boat anglers, we distributed a limited number of log books to the captains and attempted to interview the anglers when they returned to the docks. The dockside interviews proved impractical. However, we are getting encouraging results from the log book returns.

We designed a mail survey which proved to be the best sampling method for private boat anglers. We identified the owner of a particular boat by recording his registration number as he passed an observation point and then checking with the State Marine Police to see who owned the boat. Then we mailed questionnaires to 196

Figure 16.--Diver biologist examining development of attached growth on an artificial reef.

boat owners. We received completed questionnaires from over 80 percent of the boat owners sampled. Errors introduced from non-response were minimal--a follow-up survey differed by only 0.07 fish per hour in the estimate of fish per angler hour and 4 percent in the number of unsuccessful anglers. We are using aerial surveys to estimate total angling pressure in the test area.

Chester Buchanan and Richard Stone

Ecology of fish populations of artificial reefs

We supplemented SCUBA observations with longlining (Figure 17) on the Atlantic Beach, N. Y., reef and the adjacent flat sand bottom in our quantitative comparison of artificial reef with natural habitat. We caught numbers of migrant cod (Figure 18) on clam-baited hooks along with tautog, longhorn sculpin, little skate, spiny dogfish, and goosefish. Stomach contents of the cod indicated that they inhabit the reefs as well as the adjacent flat bottom.

By studying feeding habits, we learned more about the dependency of black sea bass on our South Carolina reef. They feed mostly during the day on free-living and attached organisms associated with the reef (amphipods and barnacles). To a lesser degree, they foraged on burrowing and demersal organisms of the

Figure 17.--Setting a baited longline on an artificial reef to gather quantitative information on reef productivity.

adjacent sand bottom. Feeding habits differed with size; smaller fish fed on amphipods and razor clams and larger fish on barnacles and crabs. Competition was high among sea bass, sheepshead, pinfish, and invertebrate predators for attached food organisms on the reef. In New Jersey, adults occupied new reefs immediately, even before an overlying forage population developed, suggesting an attraction to reefs based on shelter and touch-sense. We experienced highly turbid water during most diving operations in the New York Bight. The persistent disposal of solid wastes and dredge spoils, suspended in wind-generated wave surges, may produce this. We recorded low dissolved oxygen levels during summer, but did not witness any evidence of fish or shellfish mortalities, as in the autumn of 1968. These adverse water conditions may account for the paucity of demersal fishes on reefs during part of the summer, followed by repopulation from local movement when conditions improved later in the summer. A variation in oxygen concentration from 1.5 to 7 ppm from mid to late summer was associated with the change in reef fish populations.

Low light, resulting from high turbidity, may reduce feeding activity of tautog and cunner, fishes which normally browse during daytime on attached organisms. We found a heavy, relatively undisturbed, population of mussels on the upper surfaces of the Atlantic Beach reef in August with fewer tautog and cunner (Figure 19) than the dense mussel growth could support. We witnessed direct effects of low light on fishes when we made a dive on the wreck Delaware. Ambient light at mid-day was zero and under artificial lighting we found cunner wedged in crevices of the wreck where they could be touched. Their behavior was the same as that of cunner observed at night--the normal diurnal activity had been modified by turbidity-induced darkness.

At the Fire Island, N. Y., reef we made night observations to become familiar with nocturnal behavior of common reef species.

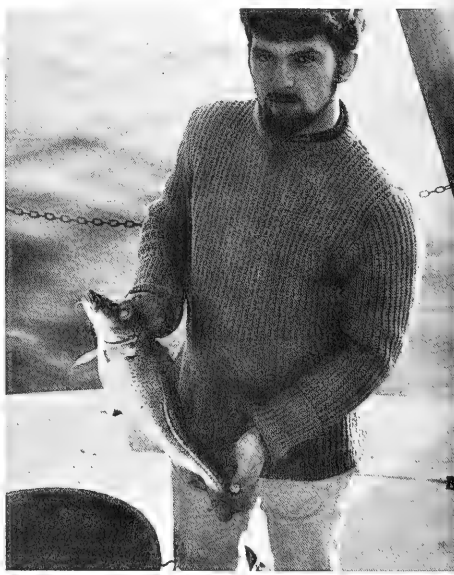


Figure 18.--Wintertime reef fishing produces cod.

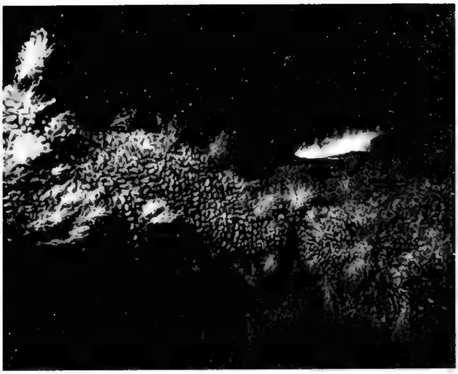


Figure 19.--The dense growth of mussels on our New York reef affords good forage.

We found aggregations of tautog and cunner wedged in spaces of sheet piling and oriented in various positions, so lethargic that sea stars were able to crawl over them (Figure 20). Although we did not witness predation of fishes by sea stars, the seasonal drop in temperature may reduce cunner activity sufficiently to allow such predation by an invertebrate organism.

In May and July we resurveyed the wreck Delaware to note long-term effects on this reef habitat from the mass mortality of 1968. The wreck fauna had re-established itself. All sizes of cunner and medium to large tautog were common and were feeding on mussels attached to upper surfaces; some large areas had been grazed bare. We found many ocean pout occupying spaces around bottom wreckage, squirrel hake nearly as numerous, but only two black sea bass along the 140-ft. long ship. Large sea anemones, especially numerous on under surfaces and inner spaces, probably survived from last year. Lobsters and rock crabs had repopulated lower portions of the wreck to nearly pre-mortality levels.

Larry Ogren and Jeffrey O'Neill

Distribution and ecology of attached marine organisms

We established two new research sites for comparative investigations of epibenthic communities. In mid-October we placed a multiple disc sampling apparatus (MDSA) near the New York State artificial reef (8 m deep) in Great South Bay, 700 yards at a bearing of 35° 30'T from the Fire Island radio mast. In cooperation with the Marine Science Research Center, State University of New York at Stony Brook, we investigated the colonization of hard surfaces (MDSA) in the intake and discharge canals of a steam-electric generating station located on Long Island Sound at Northport, New York.

Samples of epibenthic communities taken at the Shrewsbury Rocks, N. J., site over a 30-month period, provided data which allows us to predict the periods of larval settlement and colonization by species important to the

Figure 20.--At night, wrasses lie in bottom debris or wedge against solid objects. The effect of starfish is not known.

life history and success of a variety of reef-dwelling finfishes.

We are investigating the predation of the starfish, Asterias forbesi, on finfish and attached invertebrates. Our field observations and literature reviews indicate this species competes with and preys upon a variety of finfishes. We started a series of experiments to determine if sea stars are attracted to finfish by using olfactory receptors as they do with invertebrate prey.

Jack B. Pearce

Virgin Island Reef studies

This study began in February, 1968, when we conducted a SCUBA investigation of finfishes and invertebrates at Cow and Calf Reef, Jersey Bay, St. Thomas, V. I. and at Lameshur Bay, St. John, V. I. We placed a Multiple Disc Sampling Apparatus (MDSA) at Cow and Calf Reef to study larval settling, colonization, and succession of epibenthic organisms. From then to March, 1969, we collected discs monthly from the MDSA. We continued the SCUBA observations in March at the Cow and Calf Reef site and adjacent mangrove habitats in Jersey Bay.

Our analysis of disc samples indicated that tropical epibenthic communities develop much more slowly than those in temperate waters. We found only one species of gammarid amphipod, one snail, a juvenile spiny lobster, and a small unidentified crab with the discs. In contrast, discs collected after one year exposure in temperate waters of Massachusetts and New Jersey often have over 100 different species of unattached organisms associated with them.

Colonization occurred only on lower disc surfaces. Intensive grazing on the tops of the discs by reef-dwelling finfishes which apparently do not feed on the under surfaces of objects accounts for the paucity of attached or erect reef forms. The first significant settlement occurred after a 3-month period of submergence by several tube-dwelling worms on concrete and rubber discs. The oyster, Ostrea equestris, set the next month and became the dominant organism on the discs. After 12 months submergence at least six species of hard corals had colonized the discs.

We observed 68 species, representing 31 families or reef-type fishes, on the Cow and Calf Reef and adjacent area. More intensive surveys of this fringing reef could double this number. Non-piscivorous carnivores and herbivores (grunts, butterflyfish, angelfish, damselfish, parrotfish, wrasses, filefish, triggerfish, squirrelfish) dominated the fish fauna. Most piscivorous fish were non-game species, such as the trumpetfish.

The most notable feature of the Cow and Calf Reef was scarcity of predatory game fish, especially groupers and snappers. These fish, usually active at dusk or during the night, may have been missed because most of our diving occurred during the day. The reef had many caves, ledges, and pinnacles, which could function as diurnal retreats for any groupers and snappers in the area. The pelagic, wide-ranging jacks and mackerels are more difficult to observe and are seen infrequently.

In the shallow mangrove zone of Jersey Bay, St. Thomas, we recorded 20 species of fishes of 16 families. Most were juveniles of large fish found on reefs or sub-adult and adult forms of smaller fish. The shallow depths of the lagoon may preclude the larger fish from occupying this habitat. Juvenile spiny lobsters were common under the mangrove roots and rocks bordering the lagoon. The mangrove embayment affords a large protected area for the growth and development of many reef-dwelling fishes and economically important crustaceans.

Overfishing and physical alteration of stable reefs are a threat to this fragile environment. Slow to recruit new faunas, reef communities of the Virgin Islands will not survive effects of unregulated economic growth. Because the native fishing population is reef oriented, they should respond favorably to management recommendations to preserve and improve their catch, both for recreation and food.

Jack Pearce and Larry Ogren

COOPERATIVE PROGRAMS

Oil pollution

Pearce and Ogren returned to San Juan, P. R., in March and re-visited the intertidal and sublittoral environments. They had surveyed one year earlier immediately after the sinking of the tanker, Ocean Eagle. There was no trace of oil on the lower intertidal zone. Except for some areas recently or chronically polluted by fresh oils the sandy beaches and mangrove swamps were oil-free. Invertebrates and fishes appeared to be healthy and in abundance.

Underwater activities

Wicklund participated in a diving expedition to British Honduras with Edwin Link and Seward Johnson. There he gained experience in using a special chamber for decompression diving and experimented on rapid pressure changes on reef fishes.

Oceanic fish investigations

To facilitate investigation of bluefin tuna populations, Edmunds agreed to exchange blood and tissue samples from the western Atlantic for some from the Mediterranean collected by P. Pichot, Institut des Peches Maritimes, Laboratoire de SETE, France.

Cooperation with U. S. S. R.

Wilk was a member of the scientific party on the R/V Ecliptaka, a Russian research vessel taking part in joint U. S. A.-U. S. S. R. study of Atlantic shelf groundfish resources.

Cooperation with Japan

Edmunds was a member of the scientific party aboard Shoyo Maru, a research vessel of the Japan Fisheries Agency. He collected blood and tissue samples from billfish longlined from the central Caribbean in December.

North Carolina industrial fish

During cruises and field trips to sample sciaenids from North Carolina waters, we have been assisted by biologists from the States' Division of Commercial and Sports Fisheries.

Red Tide information panel

Mahoney and Pacheco served as contacts in an information alert on red tide outbreaks. They maintained liaison among biologists of the FWPCA, FDA, and N. J. State Health Department.

MEETINGS AND TRAINING

Walford participated in a symposium series on water pollution at Monmouth College, which was moderated by Clark. An effective citizen action group was organized as a result of the sessions.

Clark testified as expert witness in connection with effects of a proposed Hudson River Expressway in New York and to the Subcommittee on Fisheries and Wildlife Conservation of the Home Committee on Merchant Marine and Fisheries. He also accompanied Congressman Howard (N. J.) to the Waterways Experiment Station, Vicksburg, Mississippi, to view model operations of a proposed coastal inlet.

Azarovitz and Clark participated as members of the Marine Resources and Oceanography Working Group of the Interior's EROS (Earth Resources from Orbiting Satellites) program. This group gave partial funding support for Barnegat Bay radiometer studies. Azarovitz also attended the Sixth International Symposium for Remote Sensing of Environment at the University of Michigan and a workshop on Spacecraft Oceanography conducted by the Naval Oceanographic Office at the Naval Research Laboratory facilities in Washington, D. C.

Olla was an invited attendee to the 11th Annual Ethological Conference held in Rennes, France, and participated in the discussions on schooling behavior and feeding motivation. Later in the year he participated in the BSFW physiology workshop in LaCrosse, Wis.

Wicklund served on the Department's Man-in-the-Sea Committee.

In September, Kendall began a 10-month training session in LaJolla, Calif. He attends graduate courses at Scripps and study under Dr. Elbert H. Ahlstrom, a senior scientist of BCF. His work centers on identification of ichthyoplankton collected during the R/V Dolphin surveys.

Mahoney completed attendance at the Advanced Course in Fish Diseases at the BSFW Eastern Fish Disease Laboratory, Leetown, W. Va.

NARRAGANSETT MARINE GAME FISH LABORATORY
Narragansett, Rhode Island

HIGHLIGHTS

Our studies of spawning behavior and juvenile fish at Narragansett are yielding encouraging results, partially due to the fact that we have high quality water available for our sea-water flow system. Six species of marine game fishes spawned in the laboratory aquaria. Two species, scup and tautog, were induced to spawn twice within the year by controlling temperature and light.

The life history studies of larger, offshore game species such as sharks and billfishes have revealed the extensive seasonal north-south migrations undertaken by many of these fishes. We have found that part of the blue shark population migrates at least 2,000 miles between New England and Surinam, off South America. One of the highlights of the year was our first swordfish tag return. This fish was at liberty four years and was the first tagged broadbill recovered in the U. S. Atlantic waters.

We have also made progress in finding a means for differentiating marlin racial stocks. We examined about 350 white marlin and found that they exhibit three genetic polymorphisms, each of which may be a racially significant character.

ECOLOGY OF OCEAN GAME FISH

Our studies of life histories and migrations have yielded new information on large game species. In 1969, we tagged 1,775 sharks (18 species) and recovered 39 tags (Table 1). Since the program started in 1963, sportsmen have assisted us by tagging nearly 8,000 sharks (Figure 1) of which 217 (2.7 percent) have been recovered.

We have completed most phases of our field work and in the coming year plan to analyze all past data and prepare it for publication.

Blue shark. Five blue sharks recovered in 1969 migrated south from New England to tropical waters. The fastest rate of travel was 27 miles per day for a shark tagged near Block Canyon in September and recaptured off Venezuela in December (1,720 miles in 64 days).

Recaptures from the Caribbean area strengthened our hypothesis that this species makes extensive seasonal north-south migrations (Figure 2) similar to those proposed for white marlin and possibly for swordfish, mako, and other pelagic species.

We can show several migratory patterns for different segments of the blue shark population: 1) a movement of 300-400 miles by adult males and juveniles of both sexes between wintering grounds near the Gulf Stream, and summer grounds along the continental shelf north of 40° lat., an inshore passage probably related to feeding; 2) an offshore migration of over 700 miles by juvenile females from the coastal zone to beyond the Gulf Stream; and 3) a north-south offshore migration by part of the male population that extends at least 2,000 miles between New England and Surinam, South America.

Table 1.--Summary of shark tagging and recapture data, 1969

Species	Number tagged	Number recovered	Maximum days at liberty	Maximum distance traveled
Sandbar	142	3	1605	155
Blue	1402	31	1010	2000
Mako	20	1	56	320
Black Tip	17	2	161	800
Sharpnose	7	1	1	0
Smooth Dogfish	5	1	549	217
Other	172	0	0	0
Total	1765	39		

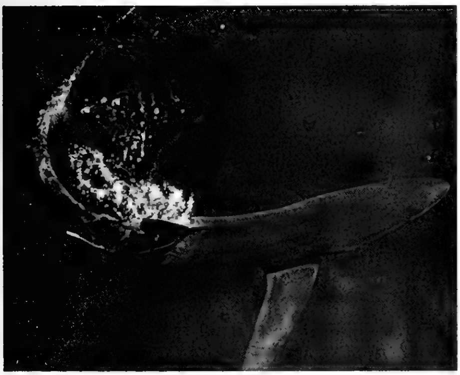


Figure 1.--A blue shark released bearing a dart tag.

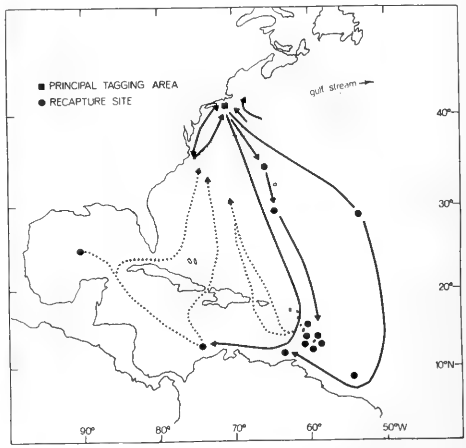


Figure 2.--Migrations of the blue shark intimated from long-range recaptures. Probable return routes are indicated by dotted pathways.

Sandbar shark. We had tags recovered from sandbar sharks after 3 and 4-1/2 years at liberty. An average annual growth increment of 4 cm shown by tagged juvenile sandbar sharks is in close agreement with estimates from our analysis of vertebral marks.

Our evidence from vertebral rings shows the species grows only a few inches per year and matures in 10 to 15 years. In June we succeeded in capturing small (60-80 cm) sandbar sharks in gill nets on their Virginia nursery grounds. With this additional material we completed the examination of vertebral sections from over 500 individuals.

Blacktip shark. A blacktip shark tagged 40 miles south of Pensacola, Fla., in August, 1968 was recaptured 80 miles ESE of Vera Cruz, Mexico, 161 days later. This is the first evidence of a trans-Gulf migration by any species of shark.

Swordfish. On July 4, 1969, a 500 lb. swordfish tagged 200 miles south of Montauk, N. Y., in August, 1965 was recaptured 48 miles ESE of the tagging area after nearly 4 years at liberty. This is one of five swordfish we tagged and is the first recovery of a tagged broadbill in U. S. Atlantic waters.

Dolphin. We kept three juvenile (38-45 cm) dolphin for 10, 22, and 52 days from August to October to study feeding and growth. Individuals ate up to 138 food items per day, amounting to 17 percent of their body weight, and grew at a rate of about 3 inches per month. The fish held longest ate a total of 6.8 lbs of food, converted approximately 26 percent of this to body weight and grew from about 1 lb to over 2 lbs during the period. These data supports evidence of a rapid growth rate for dolphin which reportedly can reach 40 lbs in 7 months under natural conditions.

John Casey and Charles Stillwell

CULTURE OF SALT WATER FISH

With the completion of work ensuring a seawater flow system with high quality water we were able to concentrate on observations and special studies of water characteristics and associated biota necessary to the well-being of marine game fish. In the coming year we plan to emphasize experimental work on behavior, physiology, and feeding of larval fishes.

Juvenile fish studies. We measured variations in growth of tautog and started a special series of experiments to learn effects of lighting and sheltered habitats. In another set of tanks we fed juvenile tautog, weakfish, and scup known amounts of fish and shrimp daily. Average weight gains over a 2-3 month period amounted to 2.5, 22.0 and 2.0 g. Expressed as percent increase of fish weight at the beginning of the study these gains were 31.9, 113.7, and 11.2, respectively.

Spawning behavior. A pair of tautog, kept in our large tank, spawned for two weeks in April providing us with viable eggs. In early June we added four males and seven females to compare group behavior with that of the pair. The newly added fish began spawning in three days continually for a month. The original pair of tautog resumed spawning in early September and continued until mid-November.

We collected cod from lower Narragansett Bay during November and December and held them in our large tank in water the same temperature as that of the Bay. At the time of collection, specimens were nearly ripe (Figure 3), and we stripped some of the cod for development studies of fertilized eggs. Cod began spawning in our tanks on December 15 and we collected and maintained fertilized eggs in lab aquaria (Figure 4). In cooperation with Dr. Howard Winn (U. R. I.), we installed a hydrophone to monitor any sounds cod make during spawning.

Figure 3.--Biologists regularly examined cod ovaries to determine the maturation of eggs.

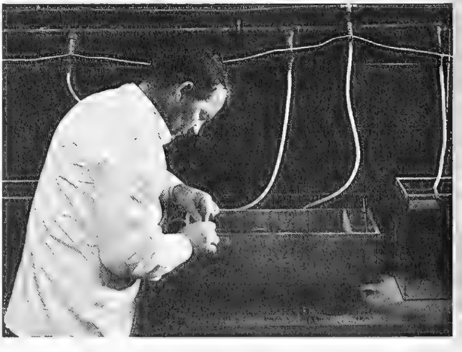


Figure 4.--Development of larval cod is followed by sampling specimens held under controlled conditions in an experimental trough.

Six scup began spawning in our tanks in late March and continued for 40 days. In September we added six more scup and in November time-phased the lighting and lowered the temperature to 15 C. for two weeks. We then lenghtened the lighted time and increased water temperature. Fish activity increased at 17 C. and in late December the scup spawned a second time within the year when temperature was 17.5 C.

Larval fish. Our second attempt to raise larval tautog beyond 10 days after hatching failed. In the trials we tested various prepared foods and investigated variations related to tank population density, water flow rate, temperature, and lighting. Seven days after hatching, larvae responded to objects in the tank, regardless of the object size or shape.

Preliminary results of a study to determine response to different light frequencies by 16-day-old cod larvae indicated a high degree to yellow, low degree to green and red, and none to blue.

We have had success in maintaining larval cod on dry prepared food and natural plankton. Preliminary observations indicated no larval response to objects placed in the aquarium.

We began monitoring development of winter flounder gonads and in the sampling found several barren adults. Some spawning occurred in our holding tanks and produced fertile eggs. We resumed rearing studies on eggs and larvae and have noted a differential development rate of the natural egg clumps with slowest rates occurring in the innermost eggs.

Connie R. Arnold and Carolyn Rogers

Effects of DDT and dieldrin on reproductive success of winter flounder

We undertook a cooperative study with the University of Massachusetts, to develop methods for sublethally dosing adult flounder with DDT, spawning, and raising the offspring of such dosed flounder. We tried the following methods of dosing: 1) incoming water, 2) food, 3) direct injection into the gonad tissue.

Water dosing proved most effective. We encountered mixed success in spawning and raised the flounder. Though spawning and egg development proceeded satisfactorily, less than 1 percent of the larvae survived to 26 days after hatching. We noted small but inconclusive differences in larval mortality between dosed and control groups. Improved techniques and materials will hopefully afford greater success in the 1970 spawning season when both DDT and dieldrin will be used.

Rod Smith and C. R. Arnold

RACIAL STUDIES

We continued to study similarities and differences of fishes on the basis of genetically-controlled protein characteristics. From this effort we are learning about the population structure of selected game fish species, such as whether particular stocks consist of a number of subpopulations. We hope to complete all present phases of work this year and analyze and publish results as appropriate.

We collected blood and tissue samples from: 452 bluefish taken along the U. S. Atlantic and Gulf coasts; 119 white marlin taken off U. S. Atlantic and Gulf coasts and from the Caribbean off Venezuela; 125 bluefin tuna along the U. S. and Canadian Atlantic coast; and 21 bluefin from Mediterranean waters. We concentrated on electrophoretic analyses of 14 blood and tissue proteins, 12 of which were enzymes (Table 2), and identified three additional genetically polymorphic characters--an oxidase in bluefin tuna and 6-PGD in both bluefin and white marlin. The oxidase system comprises two allelic genes and three electrophoretic patterns and was easily identified in fresh red blood cell (RBC) and frozen tissue. The 6-PGD enzymes, relatively unstable, show good patterns from fresh RBC, but are virtually undetectable in homogenates of frozen muscle, heart, and liver tissue. Because RBC's quickly deteriorate, 6-PGD analyses must be completed within a few days of collection.

We identified some racial differences between Rhode Island bluefish and those from North Carolina Sounds. Slight dissimilarities

Table 2.--Summary of electrophoretic separations and results, 1969

Protein	Tissue source 1/ and activity 2/						Numbers of fish examined		
	M	H	L	G	RBC	P	Bluefish	White marlin	Bluefin tuna
Enzyme:									
Lactic Dehydrogenase (LDH)	X	X	X	X			139	–	–
					X		6	12	12
Malic Dehydrogenase (MDH)	X	X	X	X			120	168	48
					X		6	98	12
Glucose-6-Phosphate Dehydrogenase (G-6-PD)	0	0	X	0			120	144	48
					0		6	6	6
Isocitric Dehydrogenase (IDH)	0	0	X	0			2	–	3
					0		–	6	6
6-Phosphogluconate Dehydrogenase (6-PGD)	0	0	0				1	1	1
					X		394	118	96
Succinic Dehydrogenase (SDH)	0	0	0	0			–	2	2
Malic	X	X	X				2	2	2
Carbonic Anhydrase (CA)					0		–	6	2
Alpha-Ketoglutarate Decarboxylase (-KG)					0		–	6	–
Alkaline Phosphatase (AP)	0	0	0	0			–	2	2
					0		–	6	–
Oxidase					X		–	–	120
Esterase	X	X	X	X			–	168	–
					X		393	134	113
						X	382	134	49
Non-enzyme:									
Homoglobin (Hb)					X		227	214	–
Transferrin (Tf)						X	–	135	134

1/ M, muscle; H, heart, L, liver; G, gonad; RBC, red blood cells; P, plasma.

2/ X - enzyme activity; 0 - little or no enzyme activity.

are evident when these groups are compared on the basis of the newly discovered 6-PGD system or the plasma esterase system we identified in 1967. Neither system alone provided proof of racial separation but the combined information is suggestive. Additional samples should give us more conclusive evidence of this difference.

Having examined only about 350 white marlin and less than 200 bluefin tuna, we must also accumulate more data on these species before conclusions about their population structures are justified. The difficulty in getting large numbers of white marlin samples from widely separated geographical areas such as the Caribbean Sea and South Atlantic Ocean is especially unfortunate and frustrating. The species exhibits at least three genetic polymorphisms: plasma esterase, plasma transferrin, and 6-PGD from RBC. Each of them may be a racially significant character and the systems are independent of each other. Collectively they represent a powerful means for differentiating marlin racial stocks.

We analyzed many fish for some proteins which exhibit genetic polymorphism in other animal species, but failed to identify such polymorphism. Some individual differences are common (e.g., 6-PGD from liver tissue of bluefish and tuna) but too complex for us to interpret with confidence. In other systems individual variations are rare (e.g., 2 of 168 marlin with an unusual pattern of MDH from heart tissue) or absent entirely (e.g., identical patterns of tissue LDH from 129 bluefish). Some enzymes (SDH, IDH, CA, KG, AP) lost activity too quickly to be detected by our procedures or may have been absent from those tissues that we examined.

Philip H. Edmunds

TECHNICAL TRANSLATIONS

During 1969, 47 assorted documents in Russian, German, Spanish, and French, totaling approximately 166,000 words, were translated into English and all scientific reports abstracted for inclusion in Sport Fishery Abstracts. Preparation is underway to transfer publication of The Division's Sport Fishery Abstracts to the Narragansett Laboratory.

Robert M. Howland

TIBURON MARINE LABORATORY

Tiburon, California
Gerald B. Talbot, Director

HIGHLIGHTS

Responsibility for conduct of the airborne sea surface temperature program is being assumed by the U. S. Coast Guard.

A prototype magnetic tape digitizer for the airborne sea surface temperature program was completed and tested.

A study was begun to determine the catch temperature for important marine game fishes.

Foreign fishing on the stocks of billfish in the eastern Pacific has resulted in fewer catches by marine game fish anglers.

Interest in the cooperative billfish tagging program has increased; however, fewer billfish were caught and fewer tagged by sportsmen during the past year.

Food studies on striped marlin show that a major portion of their diet when off southern California is anchovies.

Life history studies are continuing on the white seaperch and redtail surfperch, important marine game species occurring along the Oregon coast.

A study of the ecology and behavior of Hawaiian reef fishes was begun.

Observations along the Kona coast of the Island of Hawaii show that the much discussed coral reef destroyer, the crown-of-thorns sea star, contrary to reports, has not increased in abundance in this area.

Field sampling was completed in San Pablo Bay as part of a study to determine the effects of turbidity on Bay species.

Laboratory tests measuring the response of fish to varying levels of turbidity indicated that higher turbidity results in increased mortality.

ENVIRONMENTAL STUDIES

Recent Federal legislation has directed the U. S. Coast Guard to conduct increasing research in the field of coastal oceanography. This has resulted in the development of plans by the Coast Guard in cooperation with the Bureau of Sport Fisheries and Wildlife, to assume full responsibility for the temperature surveys in the near future. In late 1969, Coast Guard marine science technician personnel were trained in the techniques of operation of infrared equipment and in the conduct of airborne surveys, and by the year's end the Coast Guard assumed partial responsibility for this project.

The change from the fixed-wing Grumman Albatross amphibian to twin turbine helicopters at the San Diego Air Station required changes in the survey flight track and modification of the methods of mounting the infrared instrument. The limited range of the new helicopters, when compared to the fixed-wing aircraft, caused substantial reduction in the length of the survey flight track off southern California.

The development work on an automatic digitizer for the airborne radiation thermometer was completed by the U. S. Navy Fleet Numerical Weather Central, Monterey, California. The prototype unit has been flight-tested and is now in regular service on the central area survey.

The digitizer unit records sea surface temperature on magnetic tape at one-second intervals. Upon return from the survey flight, the magnetic tape data are forwarded via direct line to the Fleet Weather Central in Monterey. The data are used in computation of Navy coastal sea surface temperature charts, and the computers at Fleet Weather Central can be programmed to draw isotherm charts for each of the three coastal survey areas. This method will provide a method of obtaining electronically-computed and drawn isotherm charts of the coastal area within a short period after landing.

Sea surface temperature data obtained from August 1963 (5 years) have been analyzed and mean temperature charts drawn for each calendar month. From these charts the seasonal change in temperature gradients and isotherm patterns can be followed. A manuscript describing results of the cooperative airborne sea surface temperature program is in final draft.

Sea Surface Current study

Drift cards ballasted and sealed in plastic envelopes were dropped monthly from March 1964 through February 1966 at predetermined stations in the three airborne sea surface temperature survey areas. The results of drift card recoveries in the northern survey area (Cape Flattery, Wash. to Cape Lookout, Ore.) have been analyzed and results have been submitted for publication. Progress is being made on the analysis of the results of drift card recoveries for the central area (Point Arena to Point Sur, Calif.) and the southern area (Point Conception, Calif. to Point Salsipuedes, Mexico). Meteorological data on winds at selected locations in the two areas have been plotted and illustrations have been completed.

James L. Squire, Jr.

Pelagic fish monitoring

In 1969, six aerial fish spotters were under contract to furnish on charts a record of their flight track during fish-spotting operations with estimates of tonnage of the various species observed. These data are used in studies of the distribution and apparent abundance in near surface schooling species. This program was started in September 1962 and has provided data on the occurrences and estimates of tonnage observed for important sport and commercial species, such as yellowtail (Seriola dorsalis), Pacific barracuda (Sphyraena argentea), Pacific bonito (Sarda chiliensis), Pacific mackerel (Scomber japonicus), jack mackerel (Trachurus symmetricus), northern anchovy (Engraulis mordax), and Pacific sardine (Sardinops sagax). An index of relative apparent abundance for each species has been calculated from data collected from September 1962 through December 1966. The data have also been analyzed for diurnal variation in sightings, average size of school, and for statistics describing the magnitude of the fish spotting effort and amount of effort expended for both day and night survey flights.

A manuscript on the results of the first three years of the survey is in final draft.

Catch-temperatures of important marine game species

Using the airborne infrared radiation thermometer, we have obtained sea surface temperatures for each 10-minute longitude by latitude area having extensive sport fish catches off the southern California and central California coast. The California Department of Fish and Game records the monthly catch of each sport species caught for these same areas and these data have been furnished to us. Species under study include salmon in the central California area and Pacific bonito, Pacific barracuda, yellowtail, white seabass (Cynoscion nobilis), California halibut (Paralichthys californicus), kelp bass (Paralabrax clathratus) and sand bass (Paralabrax nebulifer), jack mackerel and Pacific mackerel in southern California. For each of these species we are determining the mean catch-temperatures and the seasonal range in catch-temperature, both for each large geographical area (such as central California or southern California) and for the important local fishing areas along the southern California and central California coast. These data are now in the process of being analyzed by computer.

James L. Squire, Jr.

COOPERATIVE TAGGING PROGRAM

The Tiburon Marine Laboratory coordinates this program as part of a cooperative effort with Woods Hole Oceanographic Institution, the International Game Fish Association, and the Mexican Department of Fisheries. Sport fishermen who enjoy catching billfishes and other large game fishes initiated the tag-and-release concept as a conservation measure and to learn about their migratory habits (Figure 1). This program began in 1963 at about the time the Japanese longline fleet moved into the Mexican sport fishing area. Tremendous catches of billfish made by the Japanese longline fishery have resulted in a 50 percent decline in catch per boat-day by the Mexican sport fishery fleet during the past five years. The number of charter boats operating in this area has increased about 14 percent during this time, but total catch has declined about 40 percent. Interest in the billfish tagging program is at an all-time high, but the number of billfish tagged has decreased during the past two years simply because fewer fish are caught.

During 1969, the following billfish were tagged: striped marlin (Makaira audax) 747; blue marlin (Makaira nigricans) 31; black marlin (Makaira indica) 40; sailfish (Istiophorus greyi) 319. In addition, 82 roosterfish (Nematistius pectoralis), 10 yellowfin tuna (Thunnus albacares) 16 yellowtail (Seriola dorsalis), 3 bonito (Sarda chiliensis), 7 dolphin (fish) (Coryphaena hippurus), and 1 each of thresher shark (Alopias vulpinus) and sheephead (Pimelometopon pulchrum), and 14 unidentified species were tagged for a total of 1,271. The three species of marlin tagged totaled 818 as compared to 1,119 in 1968 and 1,279 in 1967. The number of sailfish tagged (319) shows a decline from 432 in 1968 and 491 in 1967.

Nine tags were returned during the year. Five were from striped marlin, and one each

Figure 1.--Sailfish being tagged near Rancho Buena Vista, Baja California Sur, Mexico. Fish is played to side of boat, plastic tag is affixed using a tagging pole, and fish is released by removing hook or cutting leader.

from a black marlin, yellowfin tuna, roosterfish, and shark. The longest time between tagging and recapture was for a striped marlin tagged near Cabo San Lucas and recovered 157 days later 72 nautical miles northeast in the Gulf of California. The longest migration was 285 nautical miles from Cabo San Lucas to near the Revilla Gigedo Islands.

Interest in the tagging program increased in scope during the year. In Cairns, Australia, 39 black marlin were tagged compared to 13 the previous year. A charter boat operator in Guayaquil, Ecuador, who operates a fleet of sportfishing boats out of the port of Salinas, has begun tagging in that area.

Gerald B. Talbot

LIFE HISTORY OF FISHES

Life history of billfishes

Field investigations were primarily aimed at determining spawning time (by collection of ovarian tissue samples and examination of ovum diameters); gathering dorsal and anal fin spines for aging; and obtaining morphometric and meristic data for racial studies. Samples and data were gathered from 750 striped marlin (Makaira audax), 550 sailfish (Istiophorus greyi), and 40 blue marlin (Makaira nigricans) landed by the sport fisheries at Mazatlan, Sinaloa, and Rancho Buena Vista in Baja California Sur, Mexico (Figure 2). The sampling was conducted from late February through July and for one week in November.

The problem of aging oceanic species such as marlin is a complex one. Marlins tend to remain in waters of a relatively restricted temperature range and may grow at about the same rate the entire year; therefore, the resulting marks on the scales and the bony parts are most difficult to interpret for age. Initially, otoliths were considered as a possible method of obtaining age. However, otoliths in striped marlin are extremely small (1-2 mm) and are very difficult to locate within the bony skull. Scales are also very small and irregularly shaped. In search of better aging methods, anal and dorsal fin spines were obtained from an array of fish sizes. These fin sections show check marks, but as the fish grows, a cavity forms in the center of the spine that may erode away annular marks. The effect of this erosion or enlargement on the check marks is now being studied. Thin sections of the fin spines have been prepared in the laboratory for 250 billfish and are being examined (Figure 3) for evidence that some of the rings on the sections are annual marks and to determine if a method of distinguishing these from false annual rings can be found so that the age of these fish can be determined.

The location of striped marlin and Pacific sailfish spawning in the eastern Pacific has not been well documented although ripe marlin have been noted in Japanese catches of striped marlin near the Revilla Gigedo Islands. Our data from samples of striped marlin taken at the tip of Baja California indicate that as summer approaches, the gonad size increases markedly. However, no ripe females have been observed in the catches landed at either the tip of Baja California or at Mazatlan on the west coast of Mexico. Gonads from marlin landed at San Diego in late summer and early fall show that they are either post-spawners or in a resting stage.

Food studies were conducted by sampling stomach contents over several fishing seasons off southern California, the tip of Baja California, and the west coast of Mexico. A paper is near completion which describes in detail the food habits of 924 striped marlin caught off San Diego, Mazatlan, and Buena Vista and 197 sailfish from off Mazatlan and Buena Vista. Qualitative food data obtained in 1969 added six new fish to the list of fish species eaten by billfish in the two Mexican study areas.

While many species were consumed by billfishes, it appears from our data that they prefer certain species. In each locality one or two comprised the major portion of their diet. Off southern California, anchovies (Eugraulis mordax) were the dominant species eaten by striped marlin, with jack mackerel (Trachurus symmetricus) the second most abundant by volume. Off Mazatlan and Buena Vista, squid was the most important food item, with California

Figure 2.--Measuring the pelvic fin of a sailfish at a sport fishing dock in Mazatlan, Mexico. Morphometric data such as these are used in identifying racial stocks.

Figure 3.--Thin cross sections of spines from the dorsal and anal fins of billfish show concentric rings which may provide a means of determining age. Here, a projection of an anal spine section of 72-pound striped marlin is being examined which shows two rings and a cavity in the center of the spine which may obscure other rings.

round herring (Etrumeus acuminatus) comprising 30 percent by volume from marlin caught at Buena Vista. Squid was also a dominant food item of sailfish caught in Mexican waters. Other important species were threadfin (Polydactylus opercularis), California round herring, and cornetfish (Fistularia sp.).

Arrangements were made with the Department of Tourism for the Territory of Baja California Sur, Mexico, to obtain annual data on sport fishing effort and catches of billfishes in Baja California. Supplementing this, we have developed estimates on the amount of striped marlin and Pacific sailfish landed about the tip of Baja California and along the west coastal mainland of Mexico. These data, combined, with the records of the Japanese longline fishery, give an estimate of the total catch of striped marlin and Pacific sailfish in the northeastern Pacific.

Paul G. Wares

BEHAVIOR AND ECOLOGY OF INSHORE FISHES

Ecological relations of Hawaiian shore fishes

Our study of ecological relations of Hawaiian shore fishes began June 15. We are located on the Kona Coast of the Island of Hawaii, which offers the most favorable conditions for this type of work to be found in the Islands. In this area the bottom slopes away gradually from shore for 50 to 600 yards to where the water is 60 to 70 feet deep. Here the sea floor drops abruptly and precipitously to great depths. Thus the study area is actually a very narrow shelf on the side of a mountain, the top of which is the Island of Hawaii.

In addition to expanding our knowledge of Hawaiian shore fishes, the data obtained further broadens and refines generalizations that developed from earlier work in the tropical and warm temperate eastern Pacific. As these generalizations become better defined, many factors that influence shore fishes in all seas appear in sharper focus.

During the period June 16 to December 15, 165 separate underwater observation periods were logged--112 in daylight, 53 after dark--involving a total of over 310 hours of diving. The study is based on direct observations of activity, supplemented by examination of stomach contents. To establish differential day-night feeding activity where it occurs, collections, all by spear, have been concentrated during two periods of daylight and darkness: 1) the three hours immediately preceding sunset, and 2) the two hours immediately before first light in the morning. To further elucidate the habits of certain species, additional specimens have also been collected at other times of day and night. Thus, crepuscular fishes are also sampled immediately after morning and evening twilight, and species whose prey rapidly become unrecognizable because of digestion are also taken shortly after their feeding period begins. As of December 15, 475 specimens of 76 species had been collected for analysis of stomach contents.

Twilight activity

The transition period between day and night--morning and evening twilight--is under special study. As of December 15, observations had been made through morning twilight on 18 occasions and through evening twilight on 17 occasions. In addition to noting changes in the activity of the various fishes relative to sunrise and sunset, we have simultaneously recorded the changing levels of incident light (in foot-candles). Our photometer is not sensitive to the lower light levels (below .05 ft-c) at which many of the significant events occur. Nevertheless, we have obtained measurements when many species, for example certain wrasses, parrotfishes, and damselfishes, emerge from cover in the morning and take shelter in the evening.

Observations are still in progress, and at this time data are not ready for analysis.

Plankton-feeding fishes

The plankton-feeding fishes are a major component of the inshore fauna. None of these fishes feed on plankton during both daylight and darkness--all are either diurnal or nocturnal. Diurnal species include Chromis ovalis, C. verater, C. vanderbilti, C. leucurus, Abudefduf abdominalis, and Dascyllus albisella, all members of the damselfish family Pomacentridae; Hemitaurichthys zoster and H. thompsoni and Naso hexacanthus, both of the surgeonfish family Acanthuridae. Nocturnal species include Myripristis bernti, M. argyromus, and M. multiradiatus, all of the squirrelfish family Holocentridae; Apogon menesemus and A. snyderi both of the cardinalfish family Apogonidae; and Priacanthus cruentatus, of the bigeye family Priacanthidae.

Casual observations have indicated that the composition of the plankton, hence prey available to the plankton feeders, differs between day and night. We are investigating this possibility by taking samples of the plankton with a diver-towed net at different times of day and night at a constant depth over two particular reefs where both diurnal and nocturnal plankton feeders are active. Forty-four collections had been made by December 15. Cursory examination of the samples confirms that the composition of the plankton does indeed vary at different hours, but a complete analysis of the collections is yet to be made.

Fishes that excavate their prey

A contrast to the plankton feeders are those fishes that seek prey which are buried in the sand. In Hawaii, the most evident of these are species of the goatfish family Mullidae, which locate hidden prey with sensory barbels carried under their chin. Although one might not expect to find a day-night distinction in feeding behavior of such fishes, those studied so far have all been primarily either diurnal or nocturnal. Those feeding mostly by day include Parupeneus bifasciatus, P. multifasciatus, P. chryseydros, and P. pleurostigma. Primarily nocturnal species include Mulloidichthys samoensis, M. auriflamma, and Parupeneus porphyreus. However, the distinction is not so clear-cut as in the plankton feeders, with the diurnal species feeding to a variable, though lesser extent at night, and the nocturnal feeding to a variable, though lesser extent in daylight.

Nocturnal bottom-feeders

Bottom-feeding fishes active on the reef at night prey mostly on the many small benthic crustaceans that are themselves active in exposed locations after dark. Fishes with these nocturnal habits include Flameo sammara, Adioryx lacteoguttatus, A. tiere, A. diadema, A. xantherythrus, and Holotrachys lima, all members of the squirrelfish family Holocentridae. These fishes are all similar in appearance, a fact probably reflecting their generally similar diets. Some of the nocturnal plankton-feeders, especially Apogon menesemus and A. snyderi also feed to a lesser extent on benthic prey at night.

Diurnal bottom-feeders

In contrast to the morphological similarity among so many of the nocturnal bottom-feeders, those finding food in these same areas in daylight are extremely varied in morphology and diet.

Small, active crustaceans, the principal prey of nocturnal bottom-feeders, are far less important as prey to diurnal species. Where they are taken, as by many species of the wrasse family Labridae, they generally occupy a secondary position in a far more heterogeneous diet than regularly occurs in nocturnal species.

The many sessile organisms occurring on the reef, for example the corals, bryzoans, sponges, etc., are not generally exploited by nocturnal fishes. However, many diurnal fishes, most of them highly specialized, prey heavily on these organisms. These fishes include members of the butterflyfish family Chaetodontidae, the triggerfish family Balistidae, the pufferfish family Tetraodontidae, the filefish family Monacanthidae, and the trunkfish family Ostraciontidae.

Also included among the diurnal bottom-feeders are all of the herbivorous fishes (with the exception of a few diurnally active species that habitually take fragments of drifting vegetation from midwater). These include many species of the surgeonfish family Acanthuridae, the parrotfish family Scaridae, the damselfish family Pomacentridae, the rudderfish family Kyphosidae, and the blenny family Blenniidae. The damselfishes and the blennies, especially, show a gradation of species from carnivores to omnivores to herbivores. Consideration of the habits of these fishes provides insight into the evolution of the herbivorous diet, which is a highly evolved trait in marine fishes.

Diurnal-nocturnal coloration

Coloration of many fishes differs between day and night. We are compiling data on these color variations (Figure 4), hoping to recognize trends that suggest the significance of these variations in at least some cases. A difficulty often encountered lies in the color patterns that express stress in many species when they are held in the beam of a diving light (Figure 5). These are often difficult to distinguish from normal nocturnal patterns.

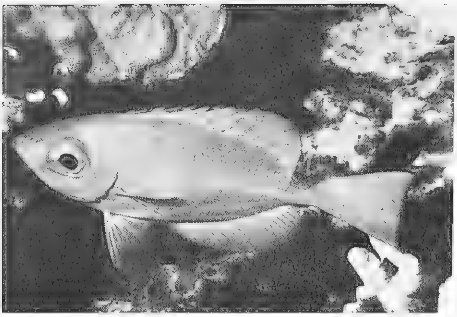


Figure 4.--Priacanthus cruentatus displaying its solid red coloration. When active in midwater at night this fish usually fades to a pale silver hue.

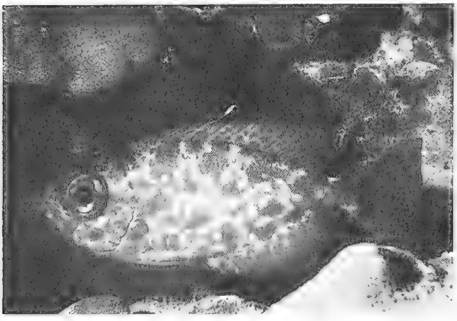


Figure 5.--This stress coloration of Priacanthus cruentatus usually appears when the fish is held under a diving light at night. This phenomenon, which occurs in many species and is usually expressed as some sort of a blotched pattern, complicates the task of recognizing true nocturnal coloration.

Observations on the Crown-of-Thorns Sea Star, Acanthaster planci, in Hawaii

During recent years the coral-eating crown-of-thorns sea star, Acanthaster planci, has become unusually numerous in certain regions of the western Pacific Ocean. Some of the ocean-oriented public, alerted by the news media to extensive damage by A. planci to the reefs of Guam and other areas, have begun looking for, and finding, concentrations of these predators on Hawaiian reefs. One major report, generating headlines in a Honolulu daily newspaper, described a vast concentration on the Kona Coast. A. planci was reported to occur "every ten feet" over the five miles between Kealakekua Bay and Homaunau. We know this report to be grossly exaggerated because the stretch of water in question is our Kona study area. If typical, it casts doubt on the many other similar reports that surged into popular print following press coverage of the Guam situation. I have not found A. planci significantly more abundant on Hawaiian reefs today than 10 years ago. Yet these reports have spawned widespread cries for control measures--generally for plans to exterminate A. planci wherever it can be found.

In the absence of good evidence that a threat to Hawaiian reefs actually exists, premature action of this sort could have undesirable results. There is no reason to believe that A. planci, a natural component of Pacific coral reef communities, has not been preying on coral for millions of years. Quite likely this sea star has contributed to the situation existing on Hawaiian reefs as we know them. Indeed a healthy situation may well require a certain number of active individuals to be present. Consider, for example, that dead coral is quickly overgrown with algae, and at this time becomes a major source of food for many herbivorous fishes, prominent on coral reefs, including some of the acanthurids, scarids and others. Wherever dead coral occurs on the reef, tooth marks of these grazing fishes are usually clearly visible where the algal covering has been scraped away (Figure 6). Thus, in providing a feeding substrate, at least some coral mortality is probably necessary for the existence of certain coral reef fishes. Other similar examples could be offered to underscore the point that measures to eradicate the sea stars should be considered with great care. At the very least, an objective study should first determine whether or not A. planci has indeed become a threat to Hawaiian reefs. A long-term solution to the problem throughout the Pacific awaits a broad study of the biology of this animal.

Figure 6.--Tooth marks of feeding herbivorous fishes, probably parrotfishes, which have scraped algae from the surface of dead coral. Note that the adjacent living coral is untouched.

Acanthaster planci in Kona

Incidental to our work with the fishes of the Kona region, we have gathered data on the occurrence and habits of A. planci. During surveys of fish populations we simultaneously recorded the incidence of this sea star, noting also the activity of each individual, as well as the species of coral that was being attacked by those that were feeding. All feeding sea stars were preying on madreporarian corals.

The observations are grouped according to three subjectively defined habitats: 1) Rocky-Reef Face, 2) Boulders, and 3) Coral-Rich Bottom.

Rocky-Reef Face.--The Kona shoreline is mostly rough lava-rock, and in many regions precipitous reefs of bare rock drop abruptly from the surface, or near surface, to water depths of 15 to 45 feet. Coral occurs on these reefs as small isolated heads of Pocillopora, or small isolated encrustations of Porites, Montipora, Pavona, Cyphastrea, or Leptastrea. This category includes the bottom at the base of these reefs, which usually are broken lava rock dotten by the same corals as the reef itself.

Boulders.--In many regions that are periodically exposed to strong surge, the sea floor is mostly bare lava boulders, which carry the same corals as the rocky-reef face.

Coral-Rich Bottom.--Where there is shelter from the waves of occasional storms at a water depths of 10 to 70 feet the bottom is heavily overgrown with corals that completely carpet the sea floor in many areas. In water depths between 10 and 35 feet, most of this coral growth consists of massive tower-like formations of Porites. In water depths between 35 and 70 feet the coral is predominantly a form of Porites that grows as finger-like branches an inch or so in diameter. Scattered in these expanses of Porites are the corals listed for the other two habitats, but in lesser abundance here.

Table 1 shows the number of surveys made during day and night in the different habitats, the average number of A. planci observed/hr., and the percent that were feeding. Table 2 shows the relative frequency with which the various forms of coral were observed among prey of A. planci.

In the rocky-reef habitat, where sea stars occur in greatest numbers (Table 1), corals grow mostly as isolated encrustations and in small heads less than 12" in diameter. These are the corals most often preyed on by A. planci in Kona (Table 2), even in the coral-rich habitat, where other corals are far more abundant. These smaller sized coral-colonies can be completely engulfed by the everted stomach of the feeding sea star, and this fact may contribute to what seems their preferred status.

Massive growths of Porites, which were prey of only 3 percent of the feeding sea stars (Table 2), and the branching finger-like colonies of Porites, which were never seen being attacked by A. planci, are by far the most abundant corals in the whole study area.

A. planci has been reported as a nocturnal animal which emerges to feed at night after being inactive under cover during the day. This is not true in the Kona region (Table 1). Most of those observed by day were feeding, and there was no evidence of increased activity at night. We noted no significant day-night difference in this animal's behavior. The relatively fewer individuals seen

Table 1.--Number of surveys made during day and night in different habitats, average number of A. planci observed per hour, and the percent that were feeding.

Habitat	No. surveys	Total time	Avg. no. sea stars observed/hr	% of total that were feeding
Rocky-Reef Face				
Day	21	16.45 hrs	9.96/hr	76%
Night	4	3.75 hrs	4.27/hr	50%
Boulders				
Day	15	10.31 hrs	2.33/hr	79%
Night	3	1.63 hrs	1.23/hr	100% (2 of 2)
Coral-Rich Bottom				
Day	25	20.03 hrs	1.04/hr	76%
Night	17	14.27 hrs	.07/hr	100% (1 of 1)
All Habitats				
Day	61	46.80 hrs	4.47/hr	76%
Night	24	19.65 hrs	.97/hr	58%

Table 2.--Relative frequency of various forms of coral observed among prey of A. planci.

Coral	% occurrence among prey of A. planci
Pocillopora	30%
Encrusting Porites	30%
Encrusting Leptastrea	19%
Encrusting Pavona	7%
Encrusting Montipora	7%
Encrusting Cyphastrea	4%
Massive Porites	3%

at night probably reflect the reduced visibility after dark, which is only partially offset by our diving lights. Despite reduced effectiveness of observations at night, we would have recognized any sharp increase in activity of A. planci if it occurred. Our observations on A. planci are continuing.

Edmund S. Hobson

San Pablo Bay study

The U. S. Army Corps of Engineers' John F. Baldwin Navigation Project calls for the deepening of the ship channel from San Francisco Bay to Stockton from the present 35-foot depth to a 45-foot depth. This would entail dredging seven channel segments totaling 68.8 miles. The initial bottom spoil material would total 84.5 million cubic yards. Approximately 8.7 million cubic yards would be disposed at sea and 20.9 million cubic yards in San Francisco and San Pablo Bays. Between Martinez and Stockton, about 54.9 million cubic yards would be disposed on 26 land and shallow water sites varying from 15 to 1,150 acres.

The Bureau of Sport Fisheries and Wildlife's report on the project, dated November 1963, recommended that a study be accomplished prior to project construction to determine the impact this project might have on fish and wildlife. Part of this study has been completed by this laboratory in cooperation with the Division of River Basin Studies for the U. S. Army Corps of Engineers.

The purpose of the study is to determine the relative abundance of marine organisms at selected sites within San Pablo Bay, the rate of natural rehabilitation of bottom fauna in areas recently disturbed by dredging and spoiling operations, and the gross effects of turbidity on fish life.

Sampling began in September 1967 at 12 stations in San Pablo Bay from the Richmond-San Rafael Bridge to Mare Island (Figure 7). These sites were selected so that dredged channel areas, spoil areas, and unspoiled areas would be sampled. In May and July 1968, three additional sampling sites were established to monitor dredging in Point San Pablo harbor and associated spoiling operations near Marin Island. Sampling methods included trawling for demersal fish, dredging for benthic organisms, and collecting water samples for oxygen, salinity, temperature, pH, and turbidity determinations (Figures 8 and 9). Field sampling was terminated August 1969. Normal seasonal fluctuations were evident in all physical parameters of Bay water measured. The highest levels of dissolved oxygen, pH, and turbidity occurred during winter and spring. Chlorosity and temperature levels were highest during summer and fall. Water samples taken after the spoil material had been dumped from a barge at the Marin Island spoil sites (stations 6A, 6B) during July 1968 were analyzed for hydrogen sulfide, dissolved oxygen, and turbidity. Hydrogen sulfide was not detected in any of the water samples and dissolved oxygen levels remained stable during the spoiling operation. The highest turbidity level measured immediately after the spoil material was released was 375 Jackson Turbidity Units (JTU).

The same procedure was again followed in December 1968 when a corps of Engineers' hopper dredge was maintenance dredging in the Pinole Shoal Channel. This time, the water samples were taken during the release of the spoil material. The highest turbidity level recorded at the associated spoil site (station 3A) was 2,000 JTU, taken from a bottom water sample, which also had a dissolved oxygen content of 0.1 ppm. Additional samples taken a few moments later indicated that the oxygen level was quickly restored. This same phenomenon has been demonstrated in the laboratory. The highest surface turbidity measured at the spoil site was 875 JTU which was collected at the same time as the 2,000 JTU bottom sample. No hydrogen sulfide was detected from any of these water samples.

The collection of Bay organisms with an otter trawl and anchor dredge in San Pablo Bay over the two-year sampling period has shown seasonal fluctuations for fish and shrimp, with the highest abundance occurring during the summer and the lowest during the winter. No seasonal fluctuations were apparent for benthic organisms although a reduction in numbers was observed in 1969 which lasted until the end of field sampling in August 1969. This reduction may have been caused by the prolonged winter and heavy spring run-off of 1968-69, although the numbers of fish and shrimp were not affected. The central and south end of San Pablo Bay had a higher abundance of benthic organisms and fish which decreased in numbers towards the northeast end of the Bay. Shallow water areas had higher numbers of fish and shrimp than deep water areas, and except for dredged channel areas, the deeper water areas were higher in numbers of benthic organisms than shallow water areas. The dredged channel had significantly lower numbers of benthic organisms, demersal fish, and shrimp.

The collection of biological data before and after spoiling operations near Marin Island in July 1968 indicated that the abundance of fish and shrimp were significantly reduced, with evidence of increasing abundance after four months. However, the benthic organisms (numerical abundance, numbers of species, and species diversity index indicative of the wealth or complexity of the community) were not significantly reduced by this same spoiling operation. One reason for this could be that spoil areas are being replenished with the benthic organisms found in the spoil material.

The spoil site located near Mare Island (station 5C), which is spoiled biannually with 1,250,000 cubic yards of spoil material from the Mare Island Strait and the Pinole Shoal Channel, had, on the average, higher numbers of benthic organisms and demersal fish than at the

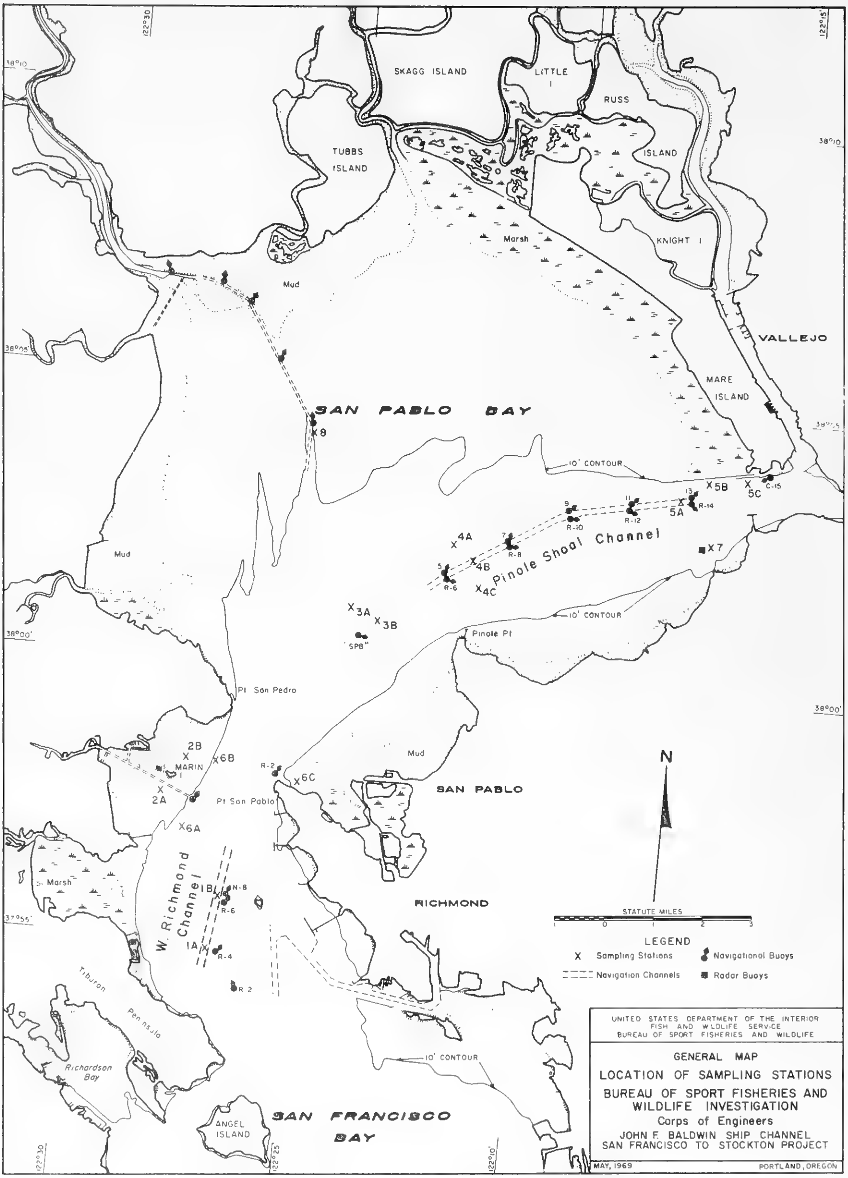


Figure 7.--Map of San Pablo Bay showing West Richmond and San Pablo Channels which the Corps of Engineers propose to dredge to a mean depth of 45 feet, and sampling stations used during the study.

Figure 8.--Biologist removing thermograph trace from continuously recording thermograph which makes week-long records of San Francisco Bay temperature at the Tiburon Marine Laboratory dock.

unspoiled area (station 5B) which was of comparable depth and chlorosity. Shrimp numbers were, however, slightly less than at the unspoiled area.

Low numbers of benthic organisms, shrimp, and demersal fish within the dredged channel areas of the Pinole Shoal Channel after dredging operations in November and December 1968 were not attributed to the dredging activities because this same occurrence was typical for the entire San Pablo Bay during this time of year.

Laboratory experiments were begun in February 1968 to test the gross effect of various turbidity levels on shiner perch (Cymatogaster aggregata), rubberlip seaperch (Rhacochilus toxotes), white seaperch (Phanerodon furcatus) striped bass (Morone saxatilus), brown rockfish (Sebastodes auriculatus), and Pacific tomcod (Microgadus proximus).

The effects of turbidity on fishes in general (measured in terms of fish-days--defined as one fish living in the test aquarium for one day, thus, ten fish living in an aquarium for one day would be equal to ten fish-days) was found to be nonsignificant between the control and 500 JTU, but to be significantly different between control, 1,500, and 2,500 JTU levels. There was a trend toward a gradual reduction in the number of fish-days and an increase in body

Figure 9.--Technician using a hydrometer to measure salinity of a sample of San Francisco Bay water.

weight loss from the control to the 2,500 JTU level. On the average, the fish in the control tank incurred the lowest percent body weight loss. All other test fish lost considerably more body weight, with the highest percent loss occurring in the highest turbidity levels. This indicates that the increased turbidity levels were either preventing the test fish from visually finding their food; caused the fish to burn more body energy from stress; or affected the fishes' well-being so that they preferred not to eat. In either case, the effect would be eventual starvation or a lowering of the fishes' body resistance to other factors which might cause death.

Brown rockfish and striped bass had a higher tolerance to high turbidity levels than shiner perch, white seaperch, and tomcod. Rubberlip seaperch, shiner perch, and tomcod were considered to have an intermediate tolerance to high turbidity levels, while the white seaperch were the most sensitive to turbidity of all the species tested.

Floyd A. Nudi

Biology of the white seaperch

There is considerable interest in such bay fishes as the surfperches for sport and commercial catch. This interest is increasing at a time when industrial demands are being made on our estuaries which might be detrimental to these fish. The white seaperch (Phanerodon furcatus) is a principal component of the sport and commercial catch of bay fishes in Oregon and California. There is a paucity of information about factors regulating its distribution and abundance.

A study of the relation of first year growth rates and abundance of white perch to abundance of principal food items is being emphasized in this investigation. The influence of first year growth rates to subsequent reproduction is also being considered. Work in the current year has consisted chiefly of sampling young perch and benthos. Four stations have been designated in Yaquina Bay, Oregon, which should represent the diversity of the Bay as an environment.

Sampling at two-week intervals commenced in late June this year. Those samples have consisted of trawling with a shrimp try-net at each station for a minimum of 100 fish per station when possible. Also included in biweekly samples are triplicate bottom dredges from each station. Samples of older perch have been taken monthly since September by using experimental gill nets.

The data on catch per unit effort for October and November of this year (Table 3) indicate that as young-of-the-year fish the white seaperch were numerically second only to the shiner perch (Cymatogaster aggregata). Comparisons of catch-per-unit effort data among stations is less reliable than comparisons of relative seasonal abundance within a station because of the physical properties of the stations.

A decline in catch per unit effort was observed between October 8 and December 3 at station 2, where all samples were taken at low tide. The number of fish caught per hour on October 8, October 20, November 17, and December 3 was 31.0, 20.6, and 0, respectively. This decline may reflect a real seasonal change in numerical strength of white seaperch during this time. Catches of other perch species decreased somewhat earlier in the season.

Growth rates of young-of-the-year perch have been calculated from changes in mean weight and from changes in weight frequency modes. Changes in weight frequency modes appear to give more reliable estimates of growth.

Monthly percentage growth rates (Table 4) were calculated by the formula:

$$R = \frac{W2 - W1}{T\left(\frac{W2 - W1}{2}\right)} \times 100$$

in which

R = percentage monthly growth rate

W1 = weight-frequency mode at beginning of period

W2 = weight-frequency mode at end of period

T = time expressed as $\left(\frac{\text{days}}{30}\right)$

After an initial lag in growth rate in July, there were highs in August through September followed by decreasing rates in later months. If the reduction in catch per unit effort is a reflection of movement of young perch from the Bay, then the decreased potential for growth by young perch may provide an explanation for such movement to other areas where the available food supply might sustain a higher growth rate. This interpretation is speculative at present and must await further analyses of food relations for verification.

To date, the food from 46 of the 225 stomachs of young perch has been examined. Five species predominated in the stomachs of fish from station 1. Three of these species are amphipods; two from the genus Corophium and one gammarid. The other two principal species are small bivalve mollusks. Although it is not yet possible to make an interpretation of the influence of food abundance on stomach contents of fish, principal stomach components appear to be benthic components. Benthos estimatics at station 1 are 1,330 per square meter for amphipods, and 450 and 145 per square meter for the two species of mollusks. Oligochaete worms were more abundant in the benthos than one of the species of mollusks but the worms were not found in stomachs. Fish

Table 3.--Catch per unit of effort by shrimp try net for all stations in Yaquina Bay, October 7 to November 19, 1969.

Species	Number of fish	Number per hour	Percent total	Perch only Percent perch
Starry flounder	390	37.7	35.4	
White seaperch	68	6.6	6.2	28.6
Pile perch	10	1.0	0.90	4.2
Striped seaperch	14	1.4	1.3	5.9
Shiner perch	146	14.1	13.2	61.3
Sand sole	217	21.0	19.7	
Staghorn sculpin	170	16.4	15.4	
Buffalo sculpin	2	0.2	0.2	
Kelp greenling	4	0.4	0.4	
Anchovy	2	0.2	0.2	
Pipefish	10	1.0	0.9	
Dungeness crab	53	5.1	4.8	
Bay shrimp	17	1.6	1.5	

Table 4.--Percentage monthly growth rates for young-of-the-year white seaperch in Yaquina Bay for July through November, 1969

Inclusive dates	Mean weight[1/] (g)	Percentage monthly growth rate
7/1 - 8/8	4.3	25.6
8/8 - 8/25	5.7	43.3
8/25 - 9/8	7.0	39.6
9/8 - 9/22	8.5	43.1
9/22 -10/7	10.2	37.1
10/7 -10/21	11.8	23.6
10/21-11/19	12.8	5.9

1/ Mean of modal weights at beginning and ending of period.

examined from station 2 contained principally a single species of the amphipod genus Corophium. The estimate of benthos from station 2 indicates levels of amphipods at 2100 per square meter and levels of bivalves at 30 and 90 per square meter.

Condition coefficients were calculated for the young-of-the-year perch in hope that they might corroborate observed growth rates. That was not the case; rather the data weakly support a generalization that condition factor increases with size. The condition factors observed were comparable with those reported for young white perch from Humboldt Bay, California.

Since the only pregnant female perch was captured in late June, I believe the spawning season ended in June. This female contained 35 near-term embryos enclosed within individual membranes within the ovisac, a condition reported for other members of the family Embiotocidae. The embryos had a mean standard length, mean total length and mean weight of 53.8, 66.9 (mm), and 3.2 (g), respectively. The scales were fully developed and the hypural plate and lateral line were evident.

A second year of the study will include samples of food and young perch from Waldport Bay, Oregon, for comparative purposes.

James R. Vanderhorst

Biology of the redtail surfperch

The redtail surfperch, Amphistichus rhodoterus (Agassiz), is perhaps the species offering the greatest recreational potential along the extensive sandy beaches of the Oregon coast. This species is also found in northern California and Washington. Published literature on the biology of this species is limited.

My major objectives were to investigate age and growth by sex, length-weight relations, relation between age and size to sexual maturity, reproductive biology, food habits, and parasites. This biological information will be useful in assessing the sport potential of this species and management needs of the species.

Gillnets, an otter trawl, and hook and line sampling between June 1967 and January 1969 indicated the redtail is available to the angler throughout the entire year in the surf and during the months of May through September in some estuaries. Rough surf conditions in winter limit angling to the most ardent of fishermen.

The catch per unit of effort in the surf fishery varied from 0.0 to 10.8 fish per angler hour with an average of 2.5 fish per angler hour. The best catch per unit of effort occurred during an incoming tide and when the surf was moderately calm. Success in an estuary (Alsea Bay) varied from 0.0 to 8.3 fish per angler hour with an average of 1.0 per angler hour. General observations of the sport fishery and gill-netting indicate redtails enter the estuary with an incoming tide. The majority of the fish leave the estuary just after the tide changes from high slack to outgoing. The fish move through the estuary in tight schools as indicated by the flurries of activity in the sport fishery, and by gill-net sets made at 15-minute intervals. The best fishing success in the estuary was found to be within an hour before and an hour after high slack tide.

The percentage of regenerated scales from a sample of 17 fish varied between 26.6 and 85.0 with a mean of 57.7. Twelve scale samples from 785 fish contained regenerated scales only and could not be aged. The age composition determined from the first scale reading of 773 surfperch is shown in Table 5. Age determinations from 108 otolith samples agreed 97 percent with readings from scales.

Table 5.--Age composition of 773 redtail surfperch as revealed by examination of scales.

Age	Number
less than 1 yr	3
1 yr	96
2 yr	130
3 yr	197
4 yr	157
5 yr	101
6 yr	57
7 yr	26
8 yr	6
9 yr	5

The body-scale relation for 773 fish was $S = 0.44\ L - 12.42$ where S = antero-lateral scale radius magnified 27 times and L equals total length. The correlation coefficient (r) for this regression was 0.95. Females grow faster than males. The longest fish was a female with a total length of 375 mm (14.8 inches) and the heaviest fish was also a female with a weight of 1,125 grams (2.47 lbs.); whereas the heaviest male weighed 695 grams (1.53 lbs.). All three fish were 9 years old. Equations for conversion of total lengths (TL) to standard length (SL) and fork length (FL) calculated from means of one-centimeter groupings of total length are $SL = 0.81\ TL - 5.29$; and $FL = 0.95\ TL - 5.09$, respectively.

A maturity index for males based on relative size of gonads reached a peak in September; however, sperm was readily emitted during late November and early December. Based on this index all males three years of age or older and 20 percent of the two-year-old males were sexually mature while all younger males were immature. Females matured later than males with none mature under three years of age. Eight percent three years old, 56 percent four years old, and all five years old or older were found to be mature. The numbers of embryos increased with the size of the parent female and varied from 1 to 39 with an average of 13 per female. The young redtails are born between August and October. Newly born redtail surfperch from four females held in the laboratory had a mean standard length (SL) of 75.78 mm and a mean weight of 5.57 grams. Because the embryos were preserved in 10 percent formalin, a shrinkage correction factor (SL fresh = SL preserved x 1.08) was calculated to eliminate shrinkage of specimens as a source of error in the study of embryonic development. This factor was determined from 12 lots of fresh embryos with mean standard lengths between 13 and 35 mm.

Food habits will be determined by frequency of occurrence and percent of total volume from preserved stomachs. These data will be further analyzed to learn differences by size of fish and differences in food habits in the surf and an estuary. Two hundred-twenty-two of 285 stomachs that have been examined to date contained a variety of food items that are being identified and categorized, while 63 stomachs were empty or contained only bait.

A sample of 357 redtail surfperch of both sexes and all ages were examined for parasites shortly after they were killed. External body parasites included the copepods Clavella sp., from fins and gills and Caligus sp., Argulus catostomi, and an unidentified species from the skin. Of particular interest was a large undescribed monogenetic trematode found on the gills. Dr. Ivan Pratt of the Zoology Department of Oregon State University is currently writing a description of this new species. This monogenetic trematode has also been found in silver and walleye surfperches. The digenetic trematode Genitocotyle acirrus was found in the intestine of every redtail examined. Nine unidentified nematodes were found in the intestine or body cavity. There was no indication that the health of the fish was affected by the parasites, but damage did occur to gill filaments and fins from the copepod infestations.

Donald E. Bennett

NATIONAL RESERVOIR RESEARCH PROGRAM

Fayetteville, Arkansas
Robert M. Jenkins, Director

HIGHLIGHTS

A nationwide compilation of reservoirs (> 500 acres) showed there were 1,320 as of December 31, 1969, totaling 8,844,000 acres at mean annual pool. During the past decade, about 314 were added, totaling 2,394,000 acres (3-1/2 percent increase in area per annum).

Partial correlation analyses involving standing crops of 32 species and species groups and 9 environmental factors provided clues of value to sport fishery management. For example: with increase in reservoir mean depth, an increase occurs in sunfish and a decrease in channel catfish, largemouth bass and white crappie crops; with increase in outlet depth, an increase in total sport fish crop; with increase in water level fluctuation, an increase in flathead catfish, black bass and white crappie and a decrease in pike and sunfish crops; with increase in storage ratio, increases in bullhead, channel catfish, largemouth bass, smallmouth bass and white crappie and decreases in flathead catfish, bluegill and longear sunfish crops.

The first segment of sport fish harvest analysis, involving sample characteristics, partial correlation and multivariable regression, was completed. Mean values of annual angler effort, success rate and harvest in 107 reservoirs were: angler-hours/acre = 71.8; angler-days/acre = 16.5; fish/hour = 0.9; pounds/hour = 0.5; pounds/acre = 24.4. The estimated mean annual harvest in all U. S. reservoirs in 1960 was 19.2 pounds per acre.

Partial correlation indicated that reservoir area, growing season and age have the most significant influence on angling. Area is negatively related to angler-hours/acre and fish caught/acre, but positively related to number of fish and pounds caught per hour. Growing season is positively related, and age of reservoir negatively related, to all angler effort and success rate parameters.

Effects of different environmental variables on both standing crop and harvest of black basses, sunfishes and catfishes were similar, but there was little agreement between total sport fish crop and total harvest.

Application of calculated curvilinear harvest regressions to the 1,320 U. S. reservoirs indicated: 1) 50 percent of the estimated total sport fish harvest (weight) occurred in 160 reservoirs over 12,000 acres in size; 2) although one-half of the total U. S. reservoir area is contained in 75 reservoirs over 24,710 acres (10,000 hectares), only 30 percent of the sport fish were harvested from them; 3) one-half of the U. S. reservoirs are from 500 to 2,000 acres in area, but accounted for only 15 percent of the total harvest; 4) 35 percent of the harvest came from 462 reservoirs 2,000 to 12,000 acres in size.

NATIONAL RESERVOIR DATA COLLECTION

A nationwide compilation of reservoirs was completed with the aid of State fishery chiefs and River Basins Studies supervisors. For our purposes, a "reservoir" is defined as an impoundment with a mean annual minimum pool of 500 acres wherein the environment is markedly influenced by engineering design and operation. Where a dam is placed at a natural lake outlet, the resulting impoundment is not considered a "reservoir" unless the area or volume is doubled. Most run-of-the-river (storage ratio < 0.01) lock and dam impoundments are excluded. Surface area is listed at mean annual pool where data are available; otherwise conservation, summer, operating or power pool area is listed.

There were about 1,320 reservoirs, totaling 8,844,000 acres, in the U. S. on December 31, 1969. Revision of the inventory data presented in ORRRC Study Report 7 ("Sport Fishing Today and Tomorrow") to provide reclassification of reservoirs to natural lakes in conformity with our definition yielded a 1960 estimate of 1,006 reservoirs, totaling 6,450,000 acres.

During the decade, about 314 new reservoirs encompassing 2,394,000 acres have been added --an annual rate of area increase of 3-1/2 percent. Major additions in area during the 1960s occurred in Texas, South Dakota, Utah, Arkansas, Oklahoma, Louisiana, Kansas, North Dakota, California and Oregon--accounting for 80 percent of the total increase.

At a continued annual increase in area of 3-1/2 percent, there will be almost 11.5 million acres at the end of 1976. This would represent an increase of 5 million acres over 1960, as predicted in ORRRC Study Report 7. However, there is some evidence that the current rate of increase will not be sustained in the 1970s. Numbers of new reservoirs will probably be added at the current rate (3 percent/ year), but average area per new reservoir will decrease.

Some characteristics of U. S. reservoirs: 1) 52 percent are 500 to 2,000 acres in size, but make up only 15 percent of the total area; 2) 75 reservoirs over 24,710 acres (10,000 hectares) comprise 50 percent of the total area; 3) one-fourth of the total area is accounted for in 16 reservoirs; 4) there are 304 reservoirs 2,000 to 5,000 acres in size, 130 between 5,000 and 10,000 acres, and 115 between 10,000 and 24,710 acres; 5) mean area equals 6,730 acres.

ENVIRONMENTAL EFFECTS ON FISH STANDING CROP

Partial correlation analyses involving standing crop of 32 species or species groups and 9 environmental variables in 7 subsamples of the 140 reservoir total sample were completed. Partial correlation eliminates the effect of those environmental variables which bias the true correlation due to their common relation with the other variables. As many of the variables used are highly correlated, partial correlation is essential to proper interpretation of the data.

Total standing crop in hydropower mainstream (storage ratio < 0.165) reservoirs is positively influenced by shore development, total dissolved solids (TDS) and growing season, and negatively by mean depth (0.05 confidence interval). These more river-like waters have relatively small differences in water level fluctuation and storage ratio, and standing crop is apparently little influenced by outlet depth and age due to high water exchange rate.

The most significant positive factor in hydropower storage reservoirs is TDS. In these more lake-like waters, storage ratio has a positive and outlet depth a negative influence (0.20 confidence interval) on standing crop. Only one of the storage reservoirs in the sample had an outlet above the top of the thermocline, precluding more definitive statements on high level vs. low level outlet effects.

The positive effect of outlet depth on crop appeared in the nonhydropower subsample. Age of reservoir also appeared as a positive influence, suggesting that flood control reservoirs are subject to more rapid eutrophication than hydropower reservoirs.

Correlation of environmental variables with the sport fish portion of the standing crop in the total sample indicated that outlet depth was the most significant influence (Table 1). Dissolved solids did not appear as a factor at the 0.20 confidence level. Surface area, fluctuation, and age of reservoir were not significant in any of the subsamples. However, some sharp differences between reservoir types are apparent. Mean depth appeared as a negative influence and

Table 1.--Logarithmic partial correlation of nine environmental variables with standing crop of sport fishes (trout, mooneyes, pike, pickerel, catfishes, bullheads, white and yellow bass, sunfishes, black basses, crappies and percids) in 140 reservoirs, and in 44 hydropower mainstream, 37 hydropower storage and 59 nonhydropower reservoirs. One symbol denotes positive or negative correlation at the 0.20 confidence level; two symbols indicate correlation at the 0.05 level; three symbols at 0.01 level.

Environmental variables	Sport fish standing crop			
	Total sample	Hydropower		Non-hydropower
		Main-stream	Storage	
Surface area				
Mean depth	-		-	
Outlet depth	+++			++
Water level fluctuation				
Storage ratio	+		++	
Shore development	+	+		+
Dissolved solids			++	
Growing season		+		
Age of reservoir				

storage ratio as a positive influence on sport fish crops only in the storage reservoirs, and outlet depth as a positive factor only in the non-hydropower reservoirs. Dissolved solids was a positive factor in hydropower storage and a negative factor in the nonhydropower reservoir subsample. Most of the storage reservoirs had low TDS content, whereas many of flood control reservoirs had mean TDS values exceeding 350 ppm, with sulfate-chloride predominating over carbonate-bicarbonate chemical types.

The hydropower subsample was redivided on the basis of presence or absence of a stable thermocline. In 55 reservoirs with a stable thermocline, partial correlation revealed significant (0.05 level) positive effects of storage ratio and TDS on total standing crop. Increase in thermocline depth has a negative effect on total crop. However, reservoirs with a thermocline typically have higher total standing crops that those without.

The analysis was expanded through partial correlation of the 9 environmental variables with 32 important species or species groups of fishes in the total sample. As all of the species did not appear in each reservoir, sample size varied from 23 to 139. Some generalizations on sport fish production influence (0.20 confidence interval) include: with increase in reservoir area, an increase in pike crop and decrease in bullheads, sunfishes and black basses; with increase in mean depth, an increase in sunfishes and decrease in channel catfish, largemouth bass and white crappie; with increase in outlet depth, an increase in combined sport fish crop; with increased water level fluctuation, an increase in flathead catfish, black bass and white crappie and a decrease in pike and sunfish crops; with increase in storage ratio (i.e., lower water exchange rate), increase in bullhead, channel catfish, largemouth and smallmouth bass and white crappie crops and decreases in flathead catfish, bluegill and longear sunfish; with increased shore development, increase in channel catfish, white bass and bluegill and decrease in redear sunfish and black crappie; with increase in TDS, increase in catfishes, white bass, green sunfish, largemouth bass and white crappie and a decrease in pike, bluegill, warmouth, and black crappie crops.

Forage fish (gizzard and threadfin shad) crops are positively influenced by increase in TDS. Gizzard shad production also responds positively to increased outlet depth, but negatively to water level fluctuation. Threadfin shad are positively influenced by growing season length, and negatively by storage ratio.

Clues to rough fish control through environmental manipulation include: mean depth is negatively related to longnose gar, carp, buffalofishes and drum crops; outlet depth is positively related to carp, carpsuckers and buffalofishes; water level fluctuation is negatively related to carpsuckers; storage ratio is negatively related to spotted sucker and redhorses; shore development is positively related to buffalofishes and carp; TDS is positively related to longnose gar, carp and carpsuckers; and age of reservoir is positively related to buffalofishes and drum and negatively to carp.

Hypothetically, largemouth bass production would be greatest in smaller, shallower reservoirs with a deep outlet, considerable annual water level fluctuation, low water exchange rate, high TDS and a long growing season. Northern pike production, in contrast, would be greatest in large reservoirs with minimum water level fluctuation, low TDS and a shorter growing season. The responses of these two species to the variables considered indicate that large crops of both could not be produced in one reservoir.

White crappie production response parallels that of the largemouth bass. White crappie crops are positively linked with outlet depth, fluctuation, storage ratio and TDS and negatively with mean depth. In contrast, the closely related black crappie responds positively to growing season and negatively to increased shore development and TDS. A decision on which of the two crappies to introduce in a new reservoir could be guided by these differences.

Correlations involving various species in all reservoirs with a stable thermocline were also computed. Some examples of apparent changes due to thermocline presence follow: largemouth bass--the negative effect of mean depth increased in significance, fluctuation appeared as a positive (0.05 level) influence, storage ratio increased and TDS decreased in positive significance. White crappie--only dissolved solids and fluctuation remained as significant positive variables; catfishes and buffalofishes--outlet depth did not appear as a positive influence; sunfishes--only growing season remained as a significant factor. Depth of thermocline had a negative effect on largemouth bass and catfish crops, and no significant effect on the other species cited.

Largemouth bass crop in reservoirs with a thermocline is positively (0.05 level) correlated with water level fluctuation, storage ratio, TDS and growing season, and negatively with both mean and thermocline depths. Knowledge of these relationships is of value in the design, operation and fishery management of impoundments, large or small.

SPORT FISH HARVEST

Harvest data were accumulated from 183 reservoirs, collated and prepared for computer analysis by staff biologist David Morais. Partial correlation and multiple regression programs were developed by Dr. James Dunn, University of Arkansas. Estimated sport harvest, by species, was available from 119 reservoirs, including 286 annual summaries. Where estimates for two or more years were available from one reservoir, a mean value was used in this analysis. Of the 119 reservoirs, 107 had data on angler effort in hours per acre and 103 in days per acre.

Mean age of the 119 reservoirs in the sample at the time of estimate was 17.4 years. Mean year when the estimates were made was 1960; ranging from 1941 through 1968. Twenty-two estimates were made before 1950; 108 from 1950 through 1959, and 156 from 1960 through 1968. Mean surface area of the reservoirs in the

Table 2.--Mean values of angler harvest, success rate and effort in 119 reservoirs (286 annual estimates). Mean reservoir area in sample equals 13,830 acres.

	Number of reservoirs	Mean	Area-weighted mean
Pounds/acre	119	24.4	14.7
Fish/acre	110	53.7	25.7
Fish/hour	107	0.8	0.85
Pounds/hour	107	0.4	0.6
Pounds/day	103	1.5	2.5
Pounds/fish	107	0.5	0.7
Angler-hours/acre	107	71.8	29.4
Angler-days/acre	103	16.5	6.4
Angler-hours/day	98	4.4	4.6

sample was 13,830 acres, compared to the U. S. mean of 6,730 acres.

The mean sport harvest of the sample was 24.4 pounds per acre; weighted by area it was 14.7 pounds per acre (Table 2). Using the regression equation derived from untransformed data (Figure 1) of pounds/acre/year on area, it is estimated that the mean harvest from all U. S. reservoirs in 1960 was 19.2 pounds/acre (17.1 kilograms/hectare). Estimated harvest from all reservoirs in 1969 totaled 170 million pounds.

Other regressions yielded the following estimates of total National reservoir harvest and effort in 1969 (based on 1960 means): Anglers expended 460 million man-hours, or 105 million man-days and caught fish at a rate of 0.37 pounds per hour, or 1.6 pounds per day. Average effort and catch rates per acre were: 52 man-hours, 11.9 man-days, 19.2 pounds, and 50 fish.

The authors of ORRRC Study Report 7 estimated mean harvest in 1960 at 17.5 pounds/acre and predicted a yield of 23 pounds/acre in 1976. Our calculations indicate a slightly higher rate of harvest in 1960, which may be attributable to our elimination of some very large waters from the "reservoir" category included in their computations. There was no significant correlation between harvest and year of census in our sample, precluding projections of future yields.

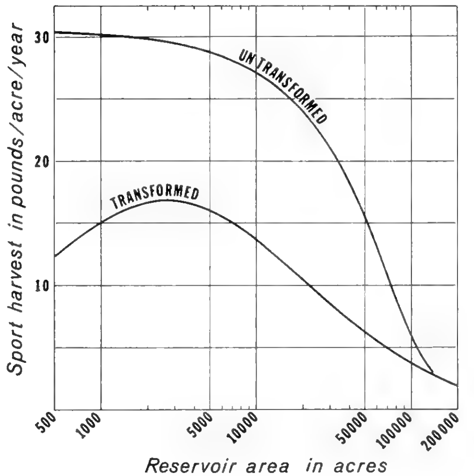


Figure 1.--Quadratic regressions of sport fish harvest on area in 119 reservoirs. For the untransformed data regression, the probability of obtaining a larger F by chance if the hypothesis of no correlation is true = 0.17. For the log transformation, the probability of a larger F = 0.01. Equations: 1) Untransformed data, pounds/acre = $0.000000001105 \text{ area}^2 - 0.00035525 \text{ area} + 30.61$; 2) log transformation, $\log(\text{pounds/acre}) = -0.2648(\log[\text{area}])^2 + 1.8154 \log(\text{area}) - 1.8848$.

as reservoir size increases, effort and harvest tend to decrease, but pounds/hour increases (Figure 2). When harvest exceeds 20 pounds/acre, catch per hour typically increases. Apparently, if catch falls below 1.5 pounds/man-day, effort drops after about 50 man-hours/acre of fishing pressure. Similarly, below a catch rate of 0.5 pounds/hour there is a tendency for effort to decrease beyond 70 man-hours/acre.

Mean harvest by species reveals highest yields of rainbow trout, crappies, sunfishes and black basses (Table 3). High trout harvests were tallied in some intensively managed reservoirs, and a substantial portion of the yield is attributable to hatchery production. The standing crop estimates (derived from summer rotenone sampling) listed are not directly comparable, as only 46 reservoirs in the two samples had both crop and harvest data. However, it suggests hypothetical mean harvests of 60 percent of the summer black bass standing crop, 25 percent of the sunfish crop, 20 percent of the catfishes and carp, and 35 percent of the crop of all sport fishes. Inadequate sampling of

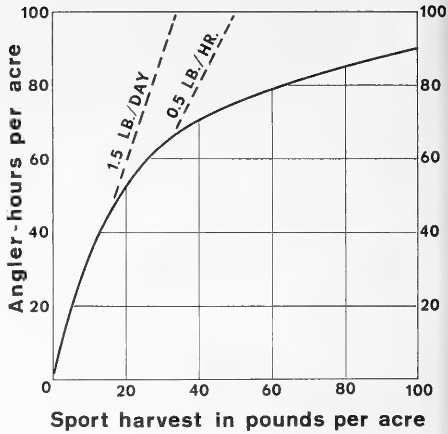


Figure 2.--Logarithmic regression of sport harvest on angler-hours in 107 reservoirs. The coefficient of determination = 0.67; i.e., two-thirds of the variability in sport harvest is explained by hours of angler effort. Commonly cited rates of harvest are plotted for comparison. Equation: $\log(\text{hours/acre}) = -0.275\ (\log/\text{pounds}\ [\text{acre}])^2 + 1.230\ \log(\text{pounds/acre}) + 0.589$.

Table 3.--Mean angler harvest compared with mean standing crop in reservoirs, by species or species groups in pounds per acre. Only 46 reservoirs in the sample supplied both crop and harvest estimates.

	Sport harvest		Standing crop
	Number of reservoirs	Pounds per acre	Pounds per acre
Black basses	92	5.3	9.0
Crappies	84	8.5	6.2
Sunfishes	86	6.1	24.8
Catfishes	69	2.2	10.5
Bullheads	55	2.7	2.2
White bass	33	2.8	3.2
Rainbow trout	36	13.7	-
Brown trout	8	1.8	-
Walleye	18	2.0	1.2
Sauger	7	1.2	-
Pike	6	0.1	1.3
Pickerel	5	1.4	1.3
Carp	43	2.5	19.3
All sport species	119	24.4	67.0

Table 4.--Simple correlation matrix of reservoir environmental variables and angler effort, success rate and total harvest. One symbol denotes positive or negative correlation at 0.20 confidence level; two symbols, the 0.05 level; and three symbols, the 0.01 level.

	Hours/ acre	Fish/ acre	Pounds/ acre	Fish/ acre	Pounds/ acre
Area	---		++	---	--
Outlet depth		---		--	-
Fluctuation		--		-	-
Storage ratio		--	-		
Growing season	++	+++		+++	+++
Age		---	-	-	-
Hours/acre			-	+++	+++
Fish/hour			+++	+++	
Pounds/hour				+	+++
Fish/acre					+++

crappies, white bass and walleye with rotenone precludes comparison of these species.

ENVIRONMENTAL EFFECTS ON HARVEST

Simple and partial correlation analyses involving harvest and 9 environmental factors revealed that only reservoir area, outlet depth, water level fluctuation, storage ratio, growing season and age were significantly related (0.20 confidence interval) to total angling effort or yield. Simple correlation indicated that area, outlet depth, growing season and age are the greatest influence on angling (Table 4). Area is negatively related (0.01 confidence interval) to angler hours/acre and fish and pounds/acre harvested, but positively linked to pounds caught/hour. Length of growing season has a highly positive effect on effort and harvest. The correlation matrix indicated positive links between all effort and yield parameters, except hours/acre vs. pounds/hour.

Partial correlation, which eliminates the bias introduced by independent variable intercorrelations, showed that area, growing season and age are the most significant factors (Table 5). As area increases, angling effort per acre decreases but success rate increases. Outlet depth, fluctuation and storage ratio are negatively related to fish and/or pounds caught per hour. As length of growing season increases yield and rate of catch increase. As age of reservoir increases, rates of harvest decrease, but there is no significant relation with angling effort. Somewhat surprisingly, no correlations were evident between shore development (relative shoreline length) or dissolved solids and angling harvest or effort.

Comparison of partial correlation results from harvest and standing crop studies showed little agreement between total harvest and crop, but close agreement between black bass, sunfish and catfish crops and environmental responses (Table 6). Both black bass and sunfish harvests and crops are negatively affected by area; black bass harvest and crop is positively influenced by growing season; sunfish harvest and crop are both negatively related to reservoir age. The crop and harvest of catfishes are both positively

Table 5.--Partial correlation of six reservoir environmental variables with angler effort and success rates in 107 reservoirs. One symbol denotes positive or negative correlation at the 0.20 confidence level; two symbols, the 0.05 level; three symbols, the 0.01 level.

	Hours/ acre	Fish/ acre	Fish/ hour	Pounds/ hour
Reservoir area	---	---	++	+++
Outlet depth			--	
Fluctuation				-
Storage ratio			--	-
Growing season	+++	+++	++	+
Age		-	---	-

Table 6.--Partial correlation of seven reservoir environmental variables vs. total standing crop and harvest of sport fishes and crop and harvest of black basses, sunfishes and catfishes. One symbol denotes positive or negative correlation at the 0.20 confidence level; two symbols, the 0.05 level; three symbols, the 0.01 level.

	Sport fishes		Black basses		Sunfishes		Catfishes	
	Crop	Harvest	Crop	Harvest	Crop	Harvest	Crop	Harvest
No. of reservoirs	139	107	135	87	136	77	124	60
Reservoir area		--	-	-	-	---		
Outlet depth	+++						+++	+
Fluctuation			+		-			
Storage ratio	+							
Dissolved solids							+++	+++
Growing season		+++	+++	+++				
Age of reservoir				-	--	---		

influenced by outlet depth and dissolved solids. No significant correlations appeared between the environmental variables and harvest of crappies.

The negative relation between total sport fish harvest and area is due, in part, to reduced access per acre and location of most huge reservoirs away from large population centers. Increased harvest resulting from longer growing seasons may be attributed to extended favorable fishing weather. Growing season is positively related to angler-hours/acre (Table 4).

Correlation and multiple regression analyses now underway involve 7 environmental factors and sport fish harvest in 4 use-type subsamples: 1) hydropower, 2) irrigation, 3) flood control, and 4) water supply and recreation reservoirs. Results should clarify some of the findings derived from total sample analysis.

NORTH CENTRAL RESERVOIR INVESTIGATIONS

Yankton, South Dakota
Norman G. Benson, Chief

HIGHLIGHTS

Because of the high water volume in the Missouri River main stem system, the U. S. Corps of Engineers increased the discharge after 1 July from Gavins Point from around 33,000 cfs in former years to over 50,000. Some effects of this increased discharge have already been identified and others will become apparent with further data analysis. In Lewis and Clark Lake, an estimated 11 million larval fish passed through the powerhouse in one 24-hour period. Temperatures were lower in all reservoirs and temperature stratification was reduced in Lakes Sharpe and Oahe. Plankton production was lower in Lakes Oahe and Sharpe. Sections of the system virtually reverted to river environment. This abrupt change in reservoir ecology further elucidates the dominating influence of water management on reservoir biological production.

With the rise of Oahe water level from 1964 to 1969, there has been a shift in distribution of the young of many fish species. Abundance has decreased in the middle and lower sections of the main reservoir and in the larger arms, and increased in the reservoir headwaters and arms. The virtual disappearance of both littoral zone and spawning habitats accounts for these changes.

Analysis of northern pike reproduction and survival in Lakes Oahe and Sharpe over the past 6 years suggests artificial propagation may be required to maintain a fishable pike population. Suitable spawning habitat appears unlikely under present water management.

We are determining the causes of differences in phytoplankton abundance by Carbon 14 production experiments. In situ fertilization experiments are used in Lake Francis Case. Chemical budgets of nitrogen and phosphorous as related to phytoplankton are being computed for Lewis and Clark Lake. These findings will enable us to relate water management to production at all trophic levels and to interpret water chemistry measurements made in the other reservoirs of the system by other agencies.

Many fish species in the Lewis and Clark Lake tailwaters grow faster than those in the reservoir proper, because of more abundant food. With our knowledge of the discharge of zooplankton, fish and benthos, feeding habits of fish, and the relations between different discharge rates and the associated biota, we should be able to predict the effects of various dis-changes on different fish species both in the tailwaters and in the reservoir.

The effects of various temperatures on the development of northern pike embryos were determined under controlled laboratory conditions. The results agreed closely with both field experiments and data on natural fish stocks.

FISH LIFE HISTORY

Spawning--Lakes Oahe and Sharpe

We continued to study spawning of common fishes in both impoundments to measure time variations in relation to changes in reservoir environment and to determine spawning success by species.

We found the spawning period of almost every species investigated has shortened during the past 6 years. During 1964-65, when Lake Oahe was rapidly filling and Lake Sharpe was attaining operational level, spawning of most species lasted from 6 weeks to over 3 months. By 1968, when Lake Oahe's maximum pool elevation had been reached, the spawning period of many species was noticeably shortened, and in 1969 it was even more so. This trend was evident at least a year earlier in Lake Sharpe. Yellow perch spawning, for example, occurred during a 2-month period in 1964 and 1965, while in 1968 it lasted 4 weeks in both impoundments and in 1969, 3 weeks. This finding suggests the reproductive potential was enhanced during the years the environment was expanding.

Some species that spawned successfully during earlier years of impoundment either have had limited spawning success or failed to spawn in recent years, with much of the egg production being resorbed. Included in this group are the pallid and shovelnose sturgeons, river carpsucker, blue sucker, northern redhorse, and channel catfish. Resorbtion of eggs appears to have resulted from lack of suitable river-type spawning habitats.

We began summarizing information collected during the past 6 years relating to spawning and survival of northern pike to make recommendations for management of this species in Lakes Oahe and Sharpe. The spawning stocks and measures of relative year-class abundance differ in these adjacent impoundments. One feature common to both populations is that spawning and survival were highly successful in the first year following impoundment. Relatively large year classes of pike were produced in Lake Oahe in 1959, the first year following impoundment, 1962, 1965, and 1969. The 1964 year class was nil, and remaining year classes were small. A relatively large year class was produced in Lake Sharpe in 1964, the first year following impoundment, but subsequent year classes were virtually absent. Relatively large year classes in Lake Oahe were produced only in those years when there was a rise in water level over vegetation during spawning and the level maintained for a time after spawning. Variations in year class size also reflected variations in fecundity and incidence of atresia within the spawning stock.

We found differences in the size at which pike reach sexual maturity in the two impoundments. The shortest mature female in Lake Oahe measured 41.8 cm, and relatively few females under 50 cm were mature. The smallest mature male was 31.5 cm, but few males under 40 cm were mature. Mature males generally averaged about 10 cm shorter than mature females at all ages. The shortest mature female in Lake Sharpe was 32.2 cm, and numerous females under 50 cm were mature. Although males in Lake Sharpe reached maturity at about the same length as the females, males averaged nearly 15 cm shorter at all ages.

We also learned that smaller, younger pike were first to occupy newly-inundated spawning grounds. Moreover, females less than 50 cm tended to spawn early in the season, while the largest females generally spawned about midseason. The tendency for larger, more fecund, females to spawn later might enhance survival, since environmental conditions usually are more favorable later in the season.

We concluded that future success of natural reproduction of northern pike in Lake Oahe will be largely dependent upon water-level management. However, because provision of suitable spawning habitat appears unlikely under present water-management, artificial propagation may eventually be required to maintain a fishable population. Prospects for a viable pike population in Lake Sharpe appear to be poor. Lack of suitable spawning and nursery habitats, along with high population levels of walleye (all ages), are the major limiting factors.

Fred June

Northern pike experiments

We installed portions of the aquarium system in our new laboratory and initiated controlled temperature experiments with embryos.

We artificially fertilized eggs and incubated them in constant temperature chambers (Figure 1) at temperatures ranging from 3 to 21 C.

Figure 1.--Part of the experimental layout for the study of the effects of temperature on northern pike embryos and yolk-sac larvae.

graduated at 3 C. intervals. Survival to hatching at 6 to 21 C. was 90 percent or above and at 3 C. about 10 percent. Time to hatching and duration of hatching were dependent upon the incubation temperature (Table 1). Hatching began in 4 days at 21 C. and in 30 days at 3 C. We transferred some of the embryos that had been incubated for 6 days at 3 C. to 12, 15, 19, and 21 C. water and found that survival to hatching among these lots ranged from 20 percent in 21 C. water to 70 percent in 12 C. water. Hatching occurred in 3 days in 21 C. water and in 5 days in 12 and 15 C. water. Embryos that had been incubated for 15 days at 3 C. did not survive transfer to 15 C. water.

Survival of unfed yolk-sac larvae ranged from less than 1 percent at 3 C. to 90 percent at 21 C. at the end of 7 days. Yolk-sac larvae hatched and developed at temperatures of 6 to 21 C. appeared to be healthy, while those incubated and hatched at 3 C. were fragile and nearly all died when disturbed or transferred to higher temperatures. We also found that survival of yolk-sac larvae that had hatched in 18 and 21 C. water before transfer to 3, 6, 9, and 12 C. water was relatively high, ranging from 75 percent at 12 C. at the end of 7 days to 90 percent at 3 and 6 C. At the end of 9 days survival was 60 percent at 3 and 6 C.

Table 1.--Time (in days) required for hatching of northern pike embryos held at various water temperatures.

Temperature (C)	Hatching time (days) Began	Completed
3	30	42
6	16	25
9	12	20
12	8	9
15	6	7
18	5	6
21	4	5

Our earlier field studies indicated that prolonged exposure of pike embryos to water temperature of near 5 C. during early developmental stages approached the lower temperature-tolerance limit for this species in Lakes Oahe and Sharpe. Our laboratory studies corroborated this finding and furthermore established that 3 C. was near lethal, whereas 6 C. was within the tolerance range of both embryos and yolk-sac larvae.

Thomas Hassler

White bass--Lewis and Clark Lake

We began studies on the reproduction potential of white bass. Pre-spawning females were captured on 15 May and the post-spawning females on 24-28 June. Ovaries were removed from fish and ovary volumes (cc) before and after spawning were measured and regressions calculated (Figure 2). Pre-spawning ovary volumes were termed potential fecundity. Post-spawning volumes were termed residual fecundity or the volume of eggs retained in the ovary after spawning. The difference between potential and residual fecundity is the volume of eggs spawned and is termed effective fecundity. There was an average of 3,904 eggs, 600 microns and larger per cc, in the pre-spawning ovaries. Eggs less than 600 μ in diameter were immature.

We made mature ova counts on 9 fish ranging from 325 to 421 mm. The number of mature ova ranged from 280,100 to 567,200. Number of ova per female was more related to fish length and weight than age.

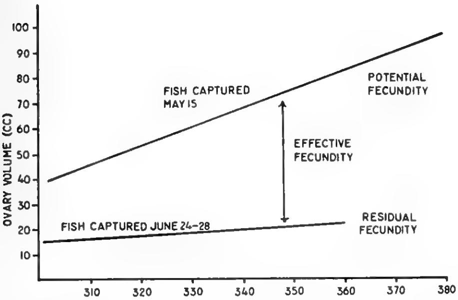


Figure 2.--Effective fecundity (number of ova spawned) by length (mm) in white bass, Lewis and Clark Lake, 1969. 1 cc of ovary contain an average of 3,904 ova.

Estimated mortality was calculated for age 0 white bass between 20 and 84 mm collected from 1964 to 1969 by trawl in the lower two-thirds of Lewis and Clark Lake (Figure 3). The relative height of each year's regression at 20 mm is an estimate of survival of young fish to a length of 20 mm. Initial survival was highest in 1969 and lowest in 1964. Relative height of the regression at 84 mm is an estimate of year-class strength by 1 September. In general, year classes abundant at 20 mm remain abundant throughout the first summer of life and year classes in which few fish are taken at 20 mm remain poor.

Richard Ruelle

Channel catfish

We determined the diet of 141 age 0 channel catfish collected from Lewis and Clark Lake. We conducted this study in cooperation with the FWPCA Laboratory in Duluth. Catfish begin feeding when approximately 15 mm long. Fish 15-20 mm long prefer zooplankton while larger fish eat both zooplankton and bottom fauna. Rotifers and algae were absent from stomachs. Diaptomus, Daphnia, and Cyclops were preferred zooplankton. Chironomids, particularly Ablabesmyia and Procladius, were preferred bottom fauna. Food electivity indices for fish 15-20 mm long showed that Diaptomus forbesi, D. ashlandi, and Daphnia pulex were highly selected. Cyclops bicuspidatus was selected by 15 mm fish but rejected by larger individuals.

Studies on the movement of catfish between Lewis and Clark Lake and the 44 mile section of the Missouri River between Fort Randall Dam and the reservoir headwaters were continued. Five hundred and thirty fish were tagged with nylon stream tags and released in the Missouri River. Nine fish were recaptured and most recoveries were upstream from location of tagging.

Charles Walburg

Walleye--Lake Francis Case

We studied spawning in May and June. Eggs were collected 5-9 May with a suction pump from near shore spawning areas at the rate of 0.64 embryos per minute of sampling. The following week only 0.06 embryos per minute were collected from the same areas. A cold wave with minimum air temperatures of 3-4 C. on three nights with accompanying strong winds occurred during the intervening weekend. No walleye larvae were taken in 212 tows with a Miller sampler during May and June. Poor hatching success for walleye in 1969 appears related to unfavorable weather conditions during the incubation period.

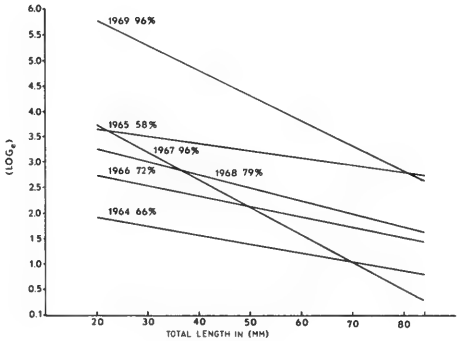


Figure 3.--Estimated mortality rates for white bass 20 to 84 mm long captured by trawl, Lewis and Clark Lake, 1964-69.

Table 2.--Food habits of age I and older walleye in percent occurrence and percent of total volume (in parentheses), Lake Francis Case, 1969. Walleye were taken with a bottom trawl in July and August, and with gill nets from September through November. t = trace

Organisms	July-Aug.	Sept.	Oct.	Nov.
Zooplankton	0(0)	0(0)	2(t)	2(0)
Fish	64(100)	41(100)	61(100)	87(100)
Yellow perch	13(9)	7(14)	3(2)	0(0)
Gizzard shad	0(0)	2(15)	8(42)	63(84)
Freshwater drum	3(12)	9(15)	13(15)	7(2)
White bass	7(18)	2(30)	3(11)	0(0)
Unidentified	55(61)	27(25)	44(32)	42(10)
Total fish examined	67	195	158	102
Percent empty stomachs	36	59	37	13

The water management of Lake Francis Case includes a 35-foot water level drawdown each year between 1 September and December. We collected 552 age I and older walleye between July and November 1969 to determine change in fish diet in relation to drawdown. Yellow perch, gizzard shad, freshwater drum, and white bass were the major diet items (Table 2). Drum was a diet item in all months, perch and white bass decreased in diet between July and November, and shad, while not found in stomachs in July-August, became the predominant food in November. The diet of 5 walleye and 35 sauger collected in November from Lewis and Clark Lake, where water levels remain relatively stable, was similar to that in Lake Francis Case.

Charles Gasaway

POPULATION DYNAMICS

Lewis and Clark Lake

Main stem Missouri River reservoirs might be termed confused environments because of the changes they undergo over the years. This reservoir was considerably different in 1969 from any year since impoundment. Reservoir releases were increased from 30,000 - 33,000 cfs to 53,000. Water exchange rates (flushing rates) were thereby increased from 7-8 days to 4-6 days. Currents created by the high water exchange affected survival of young fish in the reservoir and had an unknown influence on the vulnerability of young and adult fish to our gears during summer and fall population monitoring periods.

Losses of larval and juvenile fishes from Lewis and Clark Lake through discharge were examined on 30 days between 4 June and 13 August (Figure 4). Three metered nets were set above the powerhouse intakes to sample fishes at the surface, mid-depth, and bottom. Fishes of 15 species 5-20 cm long were taken on all but two sampling days. Species most commonly lost, in order of abundance, were freshwater drum, emerald shiner, sauger, and channel catfish (Table 3).

Estimated peak loss of drum was 10 million on 16 and 3 July, and 4 million on 14-15 July. Peak loss of 800,000 emerald shiner occurred on

Figure 4.--Gear used to collect fish above the discharge intakes of Gavins Point Powerhouse.

24 July. About 700,000 sauger were lost on 10 June, and 170,000 catfish on 28 July. Most carp, carpsucker, and white bass were lost during the third and fourth weeks of July. Except for white bass, fish loss in the discharge generally reflected species abundance in the reservoir.

Reservoir flushing rates on the 30 sample days ranged from 4.0 to 9.8, the number of days necessary to empty the reservoir at a given volume and discharge. Most fish were lost at flushing rates between 5.0 and 6.9. Lower rates were in August when most remaining fish were large enough to avoid downstream currents.

Regular collection of young-of-the-year was continued for the fifth consecutive year. Fish were sampled weekly with trawl and seine between the first week in June and second week in September. Nine species were common in collections (Table 4). Sauger and walleye are grouped together because most were less than 25 mm and hybridization makes separation of species difficult.

Strengths of the 1969 year class were estimated from comparison of annual catches (Table 4). Only white bass had a strong 1969 year class. We found increases over 1968 for yellow perch and gizzard shad. Decreases are indicated for catfish, drum, and emerald shiner. Species showing the greatest decrease in year-class abundance were all lost in large numbers in reservoir discharges (Table 3).

Adult fishes were systematically sampled for the fourth consecutive year. Catches of both gill and trap nets were about 20 percent less than in 1968 (Table 5). This decrease may be the result of increased reservoir currents during the fall of 1969. If we assume increased discharge had no effect on catch, comparison of annual catch can indicate trends in species abundance. Abundance of all species except carpsucker has decreased over the past 4 years (Table 5). Carp, carpsucker, and the buffalofishes have poor reproduction in the reservoir, and their numbers are expected to decrease. The remaining species reproduce each year but spawning success or survival of young has been only moderate.

Adult fish collected in 1966, 1967, and 1968 have been aged. Lengths and weights for most ages of fish decreased between 1966 and 1968. Greatest weight loss was experienced by carp, carpsucker, white crappie, and sauger. These species are all dependent on benthos and forage fish for food. Abundance of Hexagenia, gizzard shad and emerald shiner declined between 1966 and 1968. We believe that the decrease in food abundance caused fish growth to decline.

Charles Walburg

Gavins Point tailwater

Sampling to determine abundance and seasonal occurrence of fishes in Gavins Point Dam tailwater was concluded in April. Information on gonad development, diet, and available food was also obtained. We will relate the tailwater fish population data with that in Lewis and Clark Lake and determine the probable reasons for concentration of fish in the tailwaters.

We collected 29 fish species from the tailwaters with gill nets, but only 20 from the reservoir (Table 6). Catch-per-effort was 90 fish in the tailwaters and 27 in the reservoir. This difference in catch illustrates that fish do concentrate in the tailwaters. Blue sucker and shortnose gar are abundant in the tailwaters but

Table 3.--Estimated number (thousands) of fishes commonly lost in discharge from Lewis and Clark Lake on 30 days between 4 June and 13 August, 1969. Reservoir flushing rates are given for each sample day.

Date	Carp	River carpsucker	White bass	Channel catfish	Sauger	Freshwater drum	Emerald shiner	Flushing rate (days)
4 June	-	-	-	-	47	-	-	5.6
5	-	-	-	-	145	-	16	5.8
10	-	-	-	-	698	-	5	5.5
16	-	-	-	-	73	-	27	6.0
17	-	-	-	-	30	-	23	6.1
18	-	-	-	-	14	-	136	6.0
23	-	-	-	-	0	-	0	6.9
24	-	-	-	-	9	-	9	6.6
25	-	-	-	-	0	0	5	7.3
30	-	-	-	-	0	-	24	6.9
1 July	-	-	-	-	6	-	13	8.6
2	-	-	-	-	-	-	0	9.8
7	17	-	-	-	-	101	169	7.0
8	0	-	-	-	-	558	177	7.1
9	0	-	-	-	-	675	89	6.8
14	9	-	18	-	-	4,889	299	6.7
15	9	32	9	-	-	3,490	482	6.5
16	65	54	22	11	-	10,222	318	6.4
22	56	14	19	23	-	301	207	5.8
23	4	0	0	98	-	335	165	5.4
24	16	6	22	99	-	860	794	5.5
28	30	95	5	170	-	668	135	5.8
29	16	5	0	22	-	97	22	5.6
30	0		11	22	-	117	44	5.4
4 Aug.	10	0	-	15	-	50	10	4.5
5	-	10	-	26	-	47	10	4.6
6	-	-	-	23	-	18	56	4.6
11	-	-	-	5	-	78	16	4.4
12	-	-	-	4	-	14	0	4.3
13	-	-	-	7	-	289	35	4.3
Length range (mm)	10-26	12-28	11-35	12-35	8-26	4-54	5-32	

uncommon in the reservoir. Freshwater drum are abundant in the reservoir but uncommon in tailwaters. A number of the remaining species appear about equally abundant in both tailwaters and reservoir. These latter fishes will be examined in detail to ascertain if this portion of the tailwater population originated from the reservoir.

Preliminary findings indicate that fish concentrate in the tailwaters for spawning and/or feeding. Growth of most species collected in the tailwaters is superior to that found in Lewis and Clark Lake.

Charles Walburg

Lake Francis Case

Sampling of young-of-the-year fish to estimate relative abundance and mortality rates was continued in the lower third of the reservoir

Table 4.--Catch-effort of age 0 fish per standard haul, June-September, Lewis and Clark Lake, 1965-1969. Emerald shiner and gizzard shad taken by seine, all others by trawl.

Species	1965	1966	1967	1968	1969
Channel catfish	48	50	14	17	2
White bass	37	21	73	42	79
White crappie	23	20	10	9	8
Yellow perch	1	1	0	16	19
Sauger-walleye	9	13	3	9	10
Freshwater drum	234	138	70	133	28
Emerald shiner	55	83	49	58	32
Gizzard shad	72	67	47	14	26

Table 5.--Number of each species of fish commonly collected in September and October by gill and trap nets, Lewis and Clark Lake, 1966-1969. (Fishing effort similar among years.)

Species	Gill net				Trap net			
	1966	1967	1968	1969	1966	1967	1968	1969
Carp	222	153	168	141	241	205	165	172
River carpsucker	241	244	340	200	1,052	2,011	2,357	2,138
Smallmouth buffalo	12	19	31	21	505	565	558	255
Bigmouth buffalo	9	34	43	30	2,133	1,307	1,149	952
Channel catfish	273	194	203	168	82	67	63	45
White bass	115	45	31	25	1,010	1,378	570	642
White crappie	134	53	34	8	1,189	1,680	629	490
Sauger	415	265	303	260	142	222	144	103
Freshwater drum	377	381	368	297	1,143	2,155	1,626	1,233
Total (all species)	2,364	1,612	1,846	1,454	9,352	10,698	8,208	6,357

for the fourth consecutive year. Most river carpsucker, bigmouth buffalo, emerald shiner, and walleye were taken by 100-foot seine; most gizzard shad, white bass, white crappie, and yellow perch were taken by 27-foot bottom trawl; and most black crappie and freshwater drum by 8-foot midwater trawl. May and June levels were slightly above normal with some flooding of shore vegetation, but reproduction for most species was poor (Table 7). Only white bass reproduction was more successful than in previous years. Summer abundance of age-0 white bass has been increasing each year, but fall abundance appears similar. Few adult white bass have been captured in gill nets or in the sport fishery.

Echo sounding and midwater trawling in the limnetic zone in both 1968 and 1969 indicates there are few fish in this area. One dense fish concentration was found in this zone during the summer, and the majority of these were age-0 drum.

Charles Gasaway

Young fish stocks in Lakes Oahe and Sharpe

We measured the distribution and relative abundance of young-of-the-year fishes for the sixth consecutive year in Lake Oahe and for the third consecutive year in Lake Sharpe with the objectives of (1) assessing annual spawning

Table 6.--Common fishes collected with experimental gill nets in tailwaters of Gavins Point Dam, February 1968 to April 1969, and Lewis and Clark Lake, September and October 1968.

Species	Tailwaters	Lake
Blue sucker	20.0	2/
River carpsucker	19.0	18.5
Shortnose gar	14.8	2/
Sauger	14.1	16.4
Carp	6.7	9.1
Channel catfish	6.5	11.0
Walleye	6.0	6.6
Shovelnose sturgeon	2.0	2/
Northern redhorse	2.0	2.3
White bass	1.9	1.6
Goldeye	1.8	2.3
Freshwater drum	2/	19.6
Gizzard shad1/	2/	5.0
Bigmouth buffalo	2/	2.4
Smallmouth buffalo	2/	1.8
White crappie	2/	1.6
All other species	2.2	2/
Total catch	5,486	1,843
Total effort	61	68
Catch-effort	90	27
Total species	29	20

1/ Age 0

2/ Less than one percent

success of common species, (2) following changes in the spatial distribution of early life stages in relation to water level fluctuations, and (3) estimating mortality rates at different developmental stages. The work consisted of biweekly sampling of selected spawning and nursery areas in both impoundments with a standardized haul seine from June to September and additional sampling with an otter trawl in Lake Sharpe.

We caught 30 species of fishes in Lake Oahe and 31 in Lake Sharpe. The average catch per-unit-effort was higher for most species in Lake Oahe than in Lake Sharpe. Relative abundance indices, however, for common species in both impoundments were generally lower than in 1968 (Table 8).

Yellow perch dominated our catches in Lake Oahe for the fifth consecutive year, and white bass replaced emerald shiner as the second most abundant fish. Emerald shiner decreased noticeably in 1969, while bigmouth and smallmouth buffalos, northern pike, and carp increased. We found that years of high and low abundance of the latter four species correspond, which suggests similarities in their spawning habitat requirements.

Gizzard shad, yellow perch, emerald shiner, and walleye were the most common fishes in our catches in Lake Sharpe. Catch per unit-effort of black and white crappies and sauger suggested downward trends in 1969 while white bass apparently increased. We caught only two young-of-the-year northern pike and one black bullhead.

Table 7.--Catch per seine haul of age-0 fish during August, Lake Francis Case, 1966-1969. 1966 and 1967 catches are adjusted for station changes made in later years.

Species	1966	1967	1968	1969
Gizzard shad	0.2	12.4	25.2	6.5
Bigmouth buffalo	0.2	0.6	0	0
River carpsucker	0	0	0.4	0
Carp	0	0.4	0	0
Emerald shiner (adult)	155.5	217.4	136.7	6.1
Emerald shiner (age-0)	15.2	132.3	27.6	11.2
White bass	3.0	9.1	11.6	38.8
White crappie	21.0	16.3	0	0.1
Black crappie	15.7	2.7	0	0
Walleye	0.2	0.3	0.3	0.1
Yellow perch	4.5	9.2	1.6	1.3
Freshwater drum	3.7	4.4	2.7	3.0

Table 8.--Average numbers of common young fishes[1/] caught per standardized seine haul in Lakes Oahe and Sharpe, South Dakota, 1968-1969.

Species	Lake Oahe		Lake Sharpe	
	1968	1969	1968	1969
Yellow perch	614.3	310.4	151.3	76.6
Emerald shiner	62.0	20.7	26.5	21.4
Gizzard shad[2/]	0	0	188.3	175.6
Silvery minnow	4.2	2.6	1.3	0.3
White bass	4.2	35.4	0.2	0.7
Black crappie	15.6	3.6	1.8	0.1
Walleye	0.1	0.6	7.6	5.5
Sauger	Trace	Trace	1.3	0.3
White crappie	22.9	1.4	2.2	0.1
Number of hauls	204	225	240	120

1/ All age groups of minnows included; remainder are young-of-the-year only.

2/ Absent in Lake Oahe.

With the rise in water level of Lake Oahe over the past several years there has been an upstream shift in the distribution and areas of heaviest concentration of the young of many species. In the Cheyenne River embayment, for example, the relative abundance of common fishes was significantly higher in the upper third than in the lower and middle reaches in 1969 (Table 9), and similar trends were shown in Lake Oahe proper. Yellow perch provides an example of the changes in distribution and relative abundance that characterized a number of species in Lake Oahe (Figure 5). The relative abundance of young-of-the-year yellow perch increased from 1965 to 1967 and decreased in 1968 and 1969. Identical trends were shown in this species in the Cheyenne River embayment. Relative abundance in the lower third of Lake

Table 9.--Average numbers of common young fishes caught per standardized seine haul in the upper, middle, and lower thirds of the Cheyenne River embayment of Lake Oahe, 1969

Species	Location: Upper third	Middle third	Lower third
White bass	244.9	7.5	2.0
Bigmouth buffalo	14.7	0.1	0.1
Smallmouth buffalo	6.1	0	0
Carp	14.4	0.1	0.7
White crappie	2.1	0.5	1.5
Freshwater drum	1.6	1.1	0.1
River carpsucker	1.1	0	0
Silvery minnow	0.8	0	0
Sauger	0.2	0	0
Number of hauls	30	30	30

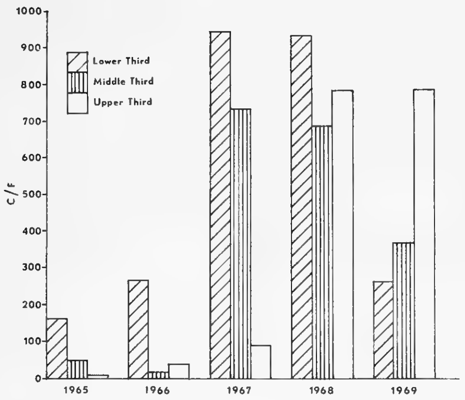


Figure 5.--Average numbers of yellow perch caught per standardized seine haul (C/f) in the lower, middle, and upper thirds of Lake Oahe, South Dakota, 1965-1969.

Oahe similarly increased each year through 1967, dropped slightly in 1968, then dropped sharply in 1969. Relative abundance in the middle third of the impoundment was notably low in 1965 and 1966, but it increased sharply in 1967 when extensive gently sloping areas in mid-reservoir became inundated. Relative abundance in the upper third of the impoundment was also low in 1965 and 1966 but increased in 1968 and 1969 when a rapid rise in water level flooded the upper reach. We conclude that the shift in distribution of young fishes simply reflects the virtual disappearance of a shallow littoral zone in much of the lower reservoir coupled with an upstream shift in the available spawning habitat for many species.

Lance Beckman

Adult fish stock in Lake Sharpe

Our studies of the adult fish stock in Lake Sharpe were continued for the fifth consecutive year with the objectives of (1) assessing changes in composition (species, age, length, and sex) in relation to the age of impoundment, (2) predicting trends in abundance of important game fish populations, and (3) elucidating the vital statistics of selected species populations. We collected biweekly samples of the adult stock with a standardized gill net at six reservoir locations from June to September and in the tailwater area from March to December.

Of 22 species taken in our nets, walleye was the most common game fish, although its relative abundance was the lowest since 1966 (Figure 6). Five postimpoundment year classes of walleye were represented, and the 1964 year class dominated for the fifth consecutive year. Incidental catches of age-I walleye suggested that

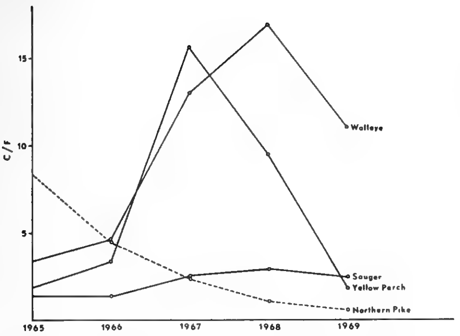


Figure 6.--Catch of some common game fishes per standard gill net (C/f) at six locations in Lake Sharpe, South Dakota, June to September, 1965-1969.

the 1968 year class was the strongest to appear since 1964. Yellow perch abundance continued to decline in 1969 and reached its lowest level since impoundment. For the third consecutive year about 85 percent of the perch catch consisted of age-II and age-III fish. Survival from age-II to age-III declined from 67 percent for the 1965 year class to 16 percent for the 1966 year class. The decline in relative abundance of age-II and age-III perch was probably due to increased predation by walleye on perch of all ages. The relative abundance of sauger remained at about the same level as in previous years, but incidental catches of age-I fish indicated that the 1968 year class probably was the smallest produced since impoundment. Our catches of northern pike have declined by about 50 percent per year since 1965 and, along with those of black bullhead, reached their lowest level in 1969.

Of rough fishes represented in our catches, six warrant comment. Relative abundance of freshwater drum continued low in 1969 (Figure 7), but the age composition gradually shifted from 21 percent postimpoundment fish in 1965 to 71 percent in 1969. The 1964 year class of carp accounted for over 80 percent of the catch each year through 1968, but very small year classes have been produced since. Thus the apparent increase in carp abundance was an artifact of changing vulnerability of the fish to our gill nets. The relative abundance of river carpsucker and goldeye have shown similar trends. Goldeye had good year classes in 1965 and in 1967, with lesser abundant year classes produced in other years, but recruitment has not kept pace with mortality of older fish. In 1968, 46 percent of the goldeye catch consisted of preimpoundment fish; in contrast, over 98 percent of the carpsucker catch consisted of preimpoundment fish. Bigmouth and smallmouth buffalos have remained at relatively low levels since 1965 with little evidence of recruitment from postimpoundment year classes.

Joseph Elrod

LIMNOLOGY

Lewis and Clark Lake

Winter--1969

The objective of this program is to determine the relations between events and conditions under the ice to subsequent conditions found during the spring and summer. Once these relations are better understood, it may be possible to predict such occurrences as the nature of spring "blooms."

We collected data on water chemistry, zooplankton, and phytoplankton under the ice from 1 January to 15 April. We developed suitable equipment and techniques for most phases of the work and collected samples biweekly from

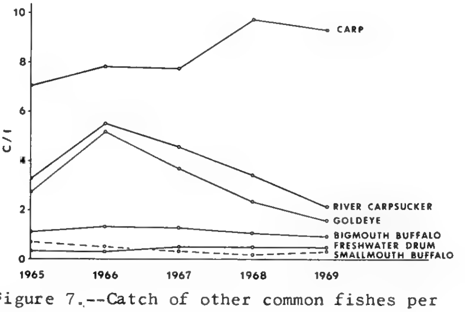


Figure 7.--Catch of other common fishes per standard gill net (C/f) at six locations in Lake Sharpe, South Dakota, June to September, 1965-1969.

one station near the dam. Total phosphorous (P) during this period averaged 0.029 mg/liter (range, 0.017-0.047). This was not significantly different from values obtained at the same station during the ice-free period of 1969 (Table 10). Soluble P averaged 0.015 mg/liter (range, 0.012-0.020). This was about twice the concentrations encountered in the lake during the following spring and summer. Nitrate (NO_3) was twice as high (average, 0.203 mg/liter; range, 0.166-0.262) under the ice than in the ice-free period.

Chlorophyll remained uniformly low (average 1.43 mg/m^3; range, 1.02-1.67) throughout the period. Because of the low chlorophyll content of the water and the reduced light conditions due to heavy snow cover, the potential for primary productivity was extremely low. Attempts to measure photosynthetic rates using changes of dissolved oxygen in light and dark bottles were unsuccessful. The more sensitive ^{14}C method would probably measure C uptake, but it was not used because of difficulties encountered in in situ incubation of samples. We recently developed methods to permit the use of ^{14}C.

Asterionella formosa was the most abundant phytoplankton found under the ice. Populations in early January were low (20/ml), they increased to a high of 850/ml on 20 February, and declined until ice left the reservoir on 14 April. Concentrations of about 100/ml were present at the end of the ice cover period. Rhodomonas, the next most numerically abundant phytoplankton taxon, were highest in January with about 500/ml present.

Cyclops bicuspidatus was the most abundant crustacean zooplankton species during the ice cover period. Individuals of the third and fourth copepodite stage were much more abundant than adults or other immature stages. Diaptomus was the only other important taxon, but densities were low.

Spring and summer--1969

In 1968, samples were taken at six stations, located equidistant along the main axis of the reservoir, to determine physical, chemical, and biological changes that occur in water as it moves down the reservoir. This sampling described the reservoir at a given time and provided information on such dynamic processes as net increase (or decrease) in plankton biomass or removal of a critical inorganic nutrient. Objectives were the same, but analyses of 1968 data resulted in a design modification for 1969. Samples in 1969 were taken: (1) in the Missouri River directly above Lewis and Clark Lake (incoming water, and termed "Headwater"); (2) from the surface (0-3 meter composite) of the reservoir at a point 3 miles from the dam over the old river channel (surface water conditions in the lower end of the reservoir and termed "Lake Surface"); (3) from a depth of 1 meter off the bottom at the same location as the lake surface sample (termed "Lake Bottom"); and (4) from the Missouri River directly downstream from the Gavins Point Dam outlet (termed "Tailwaters").

Some of the average chemical and biological conditions at the four locations for the spring and summer are shown in Table 10. A net loss of nitrate, total phosphorous and soluble phosphorous is shown as the difference in concentrations present in the Headwaters and Tailwaters samples. This means that the reservoir is retaining phosphorous at a rate which can be quantified. The nature of the retained fraction cannot be specified, but it must include incorporation into the food web and higher trophic levels. The fate of nitrate nitrogen (NO_3) is not known at this time. Changes in the form of nitrogen may occur which do not include the direct synthesis of organic matter. It is reasonable to assume, however, that uptake of NO_3 by auto trophic organisms plays a major role in this net loss of NO_3. Measurements in 1970 will include NH_4-N and NO_2-N. Experiments to determine rates of uptake of all forms of nitrogen by phytoplankton will be carried out. We will calculate a detailed phosphorous and nitrogen budget for Gavins Point Reservoir. The nutrient budget can then be related to primary production and water management practices.

Phytoplankton standing crop increases significantly between the headwaters and the dam. This is shown by the chlorophyll concentrations at the four locations (Table 10). Accompanying

Table 10.--Some chemical and biological characteristics of four sampling locations in Lewis and Clark Lake. Values are averages of all samples taken during the spring and summer of 1969.

Sampling station	NO_3-N mg/1	Total P mg/1	Soluble P mg/1	Chlorophyll mg/m^3	^{14}C productivity mg C/m^3/hr	Total zooplankton 1/ No/m^2
Headwaters	0.120	0.059	0.009	7.38	7.3	170.1
Lake surface	0.106	0.030	0.008	12.68	13.2	403.6
Lake bottom	0.115	0.032	0.008	7.49	5.6	-
Tailwaters	0.109	0.034	0.006	11.47	11.3	283.1

1/ Represents total number in 1 square meter from surface to bottom.

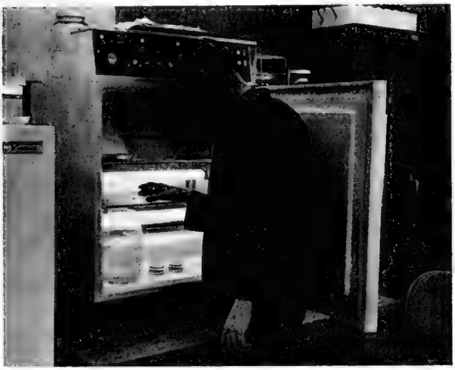


Figure 8.--Culturing Cyclops bicuspidatus under controlled temperatures.

these changes in chlorophyll is an increase in the primary productivity potential of the phytoplankton community as shown by the average ^{14}C productivity of water from each station. These figures tell an important story with respect to water management in the reservoir. Phytoplankton biomass and "potential productivity" flushed from the reservoir is lost in terms of utilization by higher trophic levels in the lake. Minor decreases in flushing rates would result in proportional increases in organic carbon synthesis within the reservoir.

Zooplankton exported from the reservoir also exceeds that imported. This means that production must be in excess of that used by predators present in the lake.

Another interesting feature is that the water discharged from the reservoir is more closely related to surface water than to bottom water. This is especially true of the phytoplankton characteristics of chlorophyll and ^{14}C uptake (Table 10). In this respect Lewis and Clark more closely resembles a natural lake than a typical "deep discharge" reservoir.

Dan Martin

Cyclops bicuspidatus

Laboratory studies of Cyclops bicuspidatus thomasi from November 1968 through April 1969 revealed instar duration, length of life cycle, behavioral characteristics, and developmental changes at 23 C. and 4.4 C. (Figure 8). Mating was observed several times and usually resulted in the production of viable eggs within 1-3 days. Most eggs were successfully hatched, and the young were reared. Females continued to form viable egg sacs for 3-4 times after initial fertilization. Egg sacs formed thereafter, without contact with males, degenerated. Unfertilized egg sacs were produced for as many as four more times, but with progressively fewer eggs.

Nauplii hatched directly from the egg sacs of the females after spending the entire prehatch period attached to the adult. This period lasted for 1-3 days at 23 C. and 7-10 days at 4.4 C. An adult female, if allowed to remain in the rearing chamber with a newly hatched group of nauplii, would eventually consume the lot. Thus the females were separated from the nauplii soon after hatching.

Naupliar duration, the period in which Cyclops are most subject to heavy mortality, was 6-10 days at 23 C., and 17-47 days at 4.4 C. There are six molts before the first copepodite stage.

Rearing from egg to adult at 23 C. required an average of 29.7 days. Only two specimens were kept alive long enough, however, to complete the cycle at 4.4 C. They attained the adult stage in 110 and 120 days, respectively. These findings will be used to interpret field measurements.

Some specimens, both adult and immature, became heavily laden with an epizooic alga (keyed to Chlorella) at both temperatures. This algal covering commonly lead to eventual immobility and death. The alga was noted only on those individuals that lived in one instar for an extended period of time. However, some Cyclops continued to molt, even though covered with algae. They discarded the algae covered with carapace and appeared to be normal.

During January-March, samples were taken through the ice near Gavins Point Dam. Samples were also collected during the open-water season in the headwaters, lower reservoir, and tailwaters. The tailwater sampling will continue during the fall and winter. The sampling will show numbers per liter for nauplii, the five copepodite stages, and adult males and females, and will explain annual population trends and fecundity rates.

Jerry Novotny

Zooplankton discharge--1964-69

Crustacean zooplankton collected from the discharge by the automatic plankton sampler was compared to the five previous years (Figure 9). Daphnia, Cyclops, and Diaptomus declined in density from 1968 to 1969. Diaptomus showed the most drastic reduction from a 6 year high in 1968. In 1969 the second highest densities were recorded for Daphnia and Diaptomus. These genera had typically low densities in Lewis and Clark Lake for the 6 year period ranging from .65 to 2.63 per liter for Diaptomus and .63 to 1.17 per liter for Daphnia.

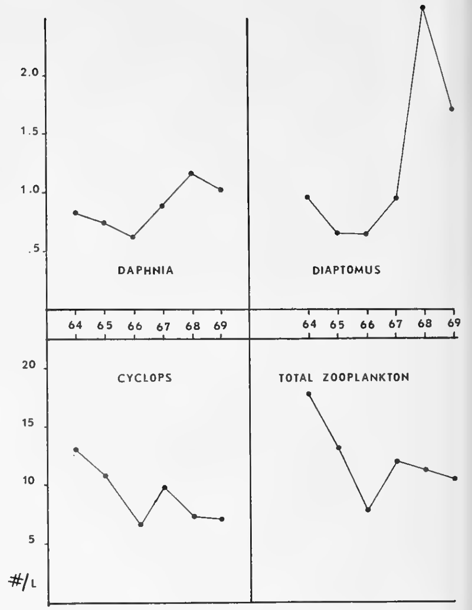


Figure 9.--Mean annual densities in number per liter of Daphnia, Diaptomus, Cyclops, and total crustacean zooplankton from Lewis and Clark Lake, 1964-1969. Samples collected every 6 hours by automatic plankton sampler.

Cyclopoids, on the other hand, continued a decline initiated in 1968 and reached the second lowest recorded yearly mean. Since Cyclops represent an average 76 percent of the zooplankton population, any fluctuation in total zooplankton numbers is directly associated with Cyclops densities.

Jerry Novotny

Benthos abundance--1963-69

We summarized standing crop data and production rates of Hexagenia in Lewis and Clark Lake from 1963 to 1969. Hexagenia was the dominant benthic invertebrate in 1963 and reached peak abundance of 6.7 g/m^2 (wet weight) in 1966. The population then declined and leveled out at around $100/m^2$ and a biomass of 4 g/m^2. The average May biomass levels from the eastern section of the reservoir from 1963 to 1969 was 5.0 g/m^2 while the October estimate from 1964 to 1968 was 4.1 g/m^2. Maximum biomass occurred in May 1966 at 7.4 g/m^2. Highest biomass levels by area were 18.8 g/m^2 in the shore area during October 1965; the lowest was 0.5 g/m^2 in the channel during September 1965. Production rates of Hexagenia follow the standing crop levels with the maximum annual rate of 20 $g/m^2/yr$. occurring in 1966. The annual turnover ratio (annual production rate/mean density) was estimated to be 3.0.

Patrick Hudson

Benthos in the limnetic zone and discharge

We studied the occurrences of benthos in the limnetic zone and the discharge in 1968 to delineate the relations among discharge rates, benthos loss through the discharge, and the biology of the benthic organisms. Miller sampler tows taken immediately in front of the discharge tunnels contained mainly chironomid larvae and pupae, Hexagenia nymphs, larval fish, ceratopogonids and water mites. Night chironomid larvae densities ranged from 8.0/m^3 in May to 0.1/m^3 in September and pupae ranged from 1.0/m^3 in August to 0.2/m^3 in July. Maximum nocturnal densities of Hexagenia reached 0.5/m^3 in May. Larval fish densities ranged from 0.3/m^3 in June to 0.1/m^3 in July.

We also studied the relations between the abundance of these migrating organisms in the water column to the biomass on the reservoir bottom and the crustacean zooplankton. Only about 4 percent of the chironomid larvae and Hexagenia nymphs on the bottom migrated into the water column at night. Length frequency distributions of migrating Hexagenia nymphs approximated those on the bottom. Although these insects were never as abundant as zooplankton, they comprised a significant portion of the plankton when zooplankton abundance was low. On 16 April 1969, along the south shore of the reservoir, a horizontal tow at mid-depth yielded 1.5 mg/m^3 (dry weight) of Hexagenia and chironomid larvae with zooplankton amounting to 7.0 mg/m^3.

Patrick Hudson

Tailwater sampling

We used Miller nets in the tailwater only to relate the collections to those taken above the dam; most sampling was done with a D-Net. This was placed 900 meters below the Gavins Point Powerhouse, and fished 24 hours biweekly. In addition to species found in the reservoir, many species endemic to the tailwaters were collected. At least 70 species of aquatic invertebrates were collected; these included 27 species of chironomids, 11 Ephemeroptera, 11 Trichoptera, 5 Corixidae, 4 Odonata, and 3 Plecoptera. Seasonal differences in drift rates were large, with the catches highest during spring and summer. Hexagenia nymphs reached the highest abundance in April and May; this is a behavioral response to population pressure in the reservoir. Trichoptera adults and pupae and chironomid pupae dominated catches from June through September as a result of normal nocturnal preemergence activity. Several species showed distinct seasonal and nocturnal drift patterns not related to emergence activities. Other species appeared in the drift because of physical disturbances (e.g., spillway releases) or maintained a constant low level of drift. Analyses indicate that this drift is the primary food of many tailwater fish.

Patrick Hudson

Systems ecology

We are going to analyze the Lewis and Clark Lake data by a compartment systems analysis program developed at the Oak Ridge National Laboratory (COMSYS). The model has been used primarily in terrestrial ecology. This stochastic program was selected because it is flexible, adapted to the type of data collected, and will work with few assumptions. The components that will be used initially will include fish (species, age, growth, and mortality rates), zooplankton, phytoplankton, and benthos. Five years of data will be used. Environmental variables such as temperature, turbidity, water level, discharge rate, and wind will be related to the flow of energy or material between compartments. Facilities at the University of South

Dakota will be used, with Dr. George Hoffman collaborating.

Norman G. Benson

Lake Francis Case

Fertilization studies

Previous work on phytoplankton standing crops, and primary productivity in this reservoir revealed extremely low biomass and autotrophic carbon assimilation during the summer months. The cause is unknown, but it is assumed that either the chemical environment limits phytoplankton growth or that the algae are being heavily grazed. Possibly both factors operate.

Experiments to determine possible factors limiting phytoplankton productivity were begun in 1969. The effects of phosphorous, nitrogen, and potassium enrichment were studied during May, June, July, and August on natural populations maintained in situ. Uptake of ^{14}C was used to measure the response of phytoplankton to various levels of inorganic enrichment. Incubating with ^{14}C of aliquots from each enriched sample was done immediately upon addition of the nutrient (day-0) and at subsequent intervals of 1, 2, and 4 days (Table 11).

Addition of potassium (K) resulted in a slight inhibition of primary production during May. This effect appeared to increase with duration of the incubation until by day-4 C uptake was about one-half of that encountered in the controls. A progressive increase in C assimilation due to K enrichment was noted during June, and a final increase of 5.6 times that in the control was obtained by day-4. After an initial inhibition in July, a slight enhancement by K was obtained. In August, inhibition occurred. An initial increase of about 2-fold was obtained but response then declined to 0.8 for the duration of the experiment.

Addition of nitrate (NO_3) resulted in inhibition during May. Nitrogen in the form of KNO_3 was added to the treatments and the results look similar to that obtained with K alone. The strong possibility thus exists that, during the May experiment, the effect of K inhibition was being measured in the N treatments rather than any

Table 11.--Response of phytoplankton to enrichment by smallest additions of potassium (K), nitrate (NO_3), and phosphate (PO_4) in Lake Francis Case, 1969. Represented as:

$$\frac{\text{average disintegrations per minute of treatment}}{\text{average disintegrations per minute of control}}$$

Month		Day 0	1	2	4
May	K	0.8	0.9	0.7	0.5
	NO_3	0.9	1.0	0.8	0.6
	PO_4	0.8	0.9	1.0	1.3
June	K	1.0	1.1	2.2	5.6
	NO_3	1.1	1.3	2.1	1.7
	PO_4	1.0	1.6	3.8	7.5
July	K	0.7	0.9	1.1	1.5
	NO_3	0.9	1.6	1.6	2.6
	PO_4	1.2	1.6	11.6	36.6
Aug.	K	1.9	0.8	0.8	0.8
	NO_3	1.9	0.9	0.9	0.7
	PO_4	2.2	2.0	4.9	6.6

effect of N *per se*. N treatment in June resulted in less enhancement than that found in K alone. Therefore, it is not possible to conclude that N alone had any enhancement effect during this month. In July, a slight increase in N effects is shown. C uptake in the N treatments exceeded that found in either the control or the K treatments by the end of the experiment. Response of K and N treatment in August was similar, again leading to the possibility that N effect may be obscured by the simultaneous addition of K.

Phsophorous had the greatest enhancement effect of any nutrients studied. In May, the increase in production was slight. Enhancement increased progressively to a high in July and continued through August. The effects of P enrichment appear to be rather easy to interpret. The P limitation is slight in spring when natural concentrations of inorganic P are highest. As natural P decreases the addition of P increased primary production. The rate of response to P additions can be followed by noting enhancement from day 0-4, and appears to increase logarithmically.

Little evidence of a NO_3 deficiency was found during the experiments. Only in July, when natural NO_3 concentrations were lowest, did NO_3 addition cause increased C uptake. Results of K enrichment were rather complicated. In some cases enhancement was noted, while in others inhibition seemed to take place. This complexity made NO_3 results difficult to interpret. Several problems encountered during 1969 have been solved.

Dan Martin

Benthos--St. Phillips Bay (Lake Francis Case)

This study, begun in 1968, was to relate benthos abundance to other chemical and biological data. Collections were made at two stations in the lower end of the reservoir. Analysis of biweekly bottom samples taken at the deep (32 m) station from May to October showed that oligochaetes dominated (95 percent), averaging $3,017/m^2$ (range, 42-$8,538/m^2$) over 11 sampling dates. Major peaks occurred in May and August-September, with minor peaks in June-July and October. Chironomid larva averaged $172/m^2$ and the rest included nematodes, mites, and ceratopogonids.

Chironomid larvae dominated the shallow (9 m) station with average density of $789/m^2$ (range, 419-$2,550/m^2$). After an initial recovery due to reflooding in May the density of chironomids decreased in June and remained fairly constant into October when receding waters or recently hatched individuals caused a significant increase. The pattern of benthos development did not follow that found in Platte Creek in 1966 where chironomids increased continually over the summer after initial reflooding and did not decrease until October.

Samples were not analyzed to species, but adult chironomids collected from emergence traps in the area have been processed. Six forms were keyed to species, and 13 genera were identified. *Harnischia darbyi* comprised 67 percent of the summer catch with *Procladius* sp. 16 percent. Other forms which were relatively abundant were species of the subfamily *Orthocladiinae* and the species *Paracladopelma* sp., *Cryptochironomus fulvus*, *Parachironomus* sp., and *Coelotanypus scapularis*.

Patrick Hudson

Benthos monitoring Platte Bay (Lake Francis Case)

We made the fourth annual fall population estimate in Platte Bay in September. We sample 4 transects at 5 foot depth intervals from 5 to 20 feet; this amounts to about 23 samples. Mean chironomid densities (No/m^2) from 1966 through 1969 were: 2,360, 1.842, 2,649, 2,544, respectively. Because of the large number of species present and single sampling date it is difficult to assess changes without critical analysis of size distribution and species composition.

Patrick Hudson

Colonization of flooded vegetation

We are studying the colonization of periphyton and invertebrates on newly flooded terrestrial vegetation in the drawdown zone.

It is important to understand how rapidly these nursery areas for young fish develop fish food. We planted sorghum-sudan hybrid in the drawdown zone in the fall of 1968, but inadequate moisture and a late drawdown resulted in poor growth. However, natural vegetation developed abundantly. The terrestrial vegetation was flooded by rising water around 13 April 1969 and was sampled for benthos and periphyton weekly from 22 April to 20 June in St. Phillips Bay. The dominant species of vegetation present at flooding were the common sunflower (Helianthus annuus), horseweed (Conyza canadensis), sedge (Carex), and wild lettuce (Lactuca virosa).

It took about 25 days after initial flooding for stems to become completely covered with a layer of periphyton. The stems were initially colonized by the sessile green filamentous algae Gongrosira which remained the base layer throughout the study period. This layer was subsequently colonized mainly by diatoms with Navicula, Diatoma, and Fragilaria succeeding each other in that order from 29 April to 29 May (Table 12). By 6 June the lowering of the water level exposed most of the plants with periphyton.

Aquatic insect development did not become significant until 40 days after flooding and was still increasing when the study was terminated. Grab samples of vegetation on the first two sampling dates contained no aquatic insects but contained semiaquatic Oligochaetes and larval forms of terrestrial Lepidoptera and Coleoptera. Collections in the following weeks showed a few chironomid larvae and egg masses. On 22 May early instars of Chironomus attenuatus became extremely abundant (Table 12). They were the most abundant invertebrate until the final sampling date when the mayfly Callibaetis and damselflies became numerous. Quantitative samples were taken in the vegetation and in a control area. The processed results will describe the development of fauna and flora on flooded vegetation.

Wave action on exposed vegetation quickly knocked down and eroded away most plant material and piled it along the shoreline and in small bays. This physical action and wave induced turbidity limited periphyton development. This turbidity and windrowed vegetation resulted in temperatures 13 C. greater along the shore and in small keys than in the main body of the reservoir on 22 May due to low water exchange rate. Carp spawning activity in the areas during May also caused high turbidity levels. Many carp eggs were found on vegetation on 22 and 29 May.

Patrick Hudson

Lakes Oahe and Sharpe

We completed seven limnological cruises on Lakes Oahe and Sharpe during which we measured water temperature, turbidity, conductivity, transparency, oxygen content, phytoplankton, and zooplankton at established stations for purposes of determining seasonal and annual variations in the reservoir environment and assessing their effect on the fish stocks. Summaries of physical and chemical data are complete: processing the plankton samples remains to be done.

Pool elevation in Lake Oahe increased from a low of 1,604 ft msl in mid-January to a maximum of 1,616 ft msl in early May and remained fairly constant through the remainder of the month. Discharges through Oahe Dam into Lake Sharpe increased during June and early July but reached record volumes during August, when there was a loss of 1.32×10^6 acre ft of water from Lake Oahe. The impact of this high discharge was most obvious in 85-mile long Lake Sharpe, where in order to maintain a relatively constant pool elevation, the rate of flow through Big Bend Dam (lower end of Lake Sharpe) equaled the inflow from Oahe Dam. Some of the immediate effects included (1) a marked decrease in zooplankton abundance, (2) a lower mean annual cycle of water temperature, and (3) a virtual absence of thermal stratification. The importance of these and other changes to the biology of the fish stocks in Lake Sharpe is being investigated but analysis of the data are too incomplete to permit an assessment at this time.

Mr. Ron Rada, a graduate student at the University of South Dakota, completed the sorting and counting of organisms in phytoplankton and zooplankton samples collected in Lake Oahe

Table 12.--Dominant invertebrates and periphytic algae found on flooded terrestrial vegetation in St. Phillips Bay, Lake Francis Case during the spring of 1969. Flooding occurred on 13 April.

Date	Periphyton	Invertebrates (*very abundant)	Maximum temperature (C)
22 April	Gongrosira	Terrestrial forms	9
29 April	Navicula Gongrosira	Terrestrial forms	10
7 May	Diatoma Gomphonema Gongrosira	Chironomid egg masses Micropsectra nigripila Cricotopus sp. Trissocladius nivoriundus	13
14 May	Diatoma Synedra Gongrosira Fragilaria	Cricotopus sp.	20
22 May	Fragilaria Synedra Gongrosira	Chironomus attenuatus* Cricotopus Trissocladius nivoriundus	27
29 May	Fragilaria Synedra Gongrosira	Chironomus attenuatus* Glyptotendipes barbipes Chironomid egg masses Aquatic beetles	22
6 June	Periphyton exposed by drawdown	Chironomus attenuatus* Glyptotendipes barbipes Corixidae	18
20 June	Periphyton exposed by drawdown	Chironomus attenuatus* Callibaetis* Damselflies* Tanypodinae	19

from 1966 to 1968. Some of the more important features shown by the phytoplankton data were (1) relatively low overall densities, combined with a general decline during the 3-year period, (2) higher densities and a greater diversity of organisms at the mid-reservoir stations, and (3) low densities and few kinds of organisms at the downstream stations. Of 54 genera of phytoplankers found in the samples, only three (Asterionella, Rhodomonas, and Cryptomonas) were consistently represented at most stations, and seven were considered to be of major importance. Completion of the phytoplankton analysis will permit us to subject all of the physical and biological data collected during the 3-year period to computer analysis.

We held two workshops in Pierre for purposes of reviewing our limnological studies in Lakes Oahe and Sharpe, discussing long-term research goals and needs, and coordinating our studies with those of other State and Federal agencies. One of the immediate results of the workshops was an exchange of data collected by the various agencies. We distributed summaries of our chemical and physical station data and will soon disseminate the plankton data.

Fred June

SOUTH CENTRAL RESERVOIR INVESTIGATIONS

Fayetteville, Arkansas
Thomas O. Duncan, Chief

HIGHLIGHTS

Gizzard shad larvae show progressive seasonal changes in vertical distribution, with concentration near the surface in late April, shifting to 5 meters in late May. Threadfin shad larvae appeared in greatest numbers along shorelines in May, but peak densities alternated between shoreline, open water surface and 5 meter samples thereafter. Both species moved to deeper water following prolonged strong winds in June.

SCUBA was used in placing and retrieving benthic fauna samplers in the littoral region of Beaver and Bull Shoals reservoirs. Fish excluders of different mesh sizes were placed around some of the samplers. Although ceramic pot samplers are not the final answer, they are well suited for comparative studies by offering a uniform dimension and functioning over a wide range of substrates.

Avoidance of bluff habitat by spawning spotted bass appears to be positively correlated with high water levels. The number of spawning spotted bass in Bull Shoals reservoir along an 800 yard bluff was much smaller in 1968 and 1969 when water levels were about 20 feet higher than in 1965, 1966, and 1967. Nests of spotted bass in coves were of expected densities suggesting more attractive spawning habitat due to inundation of dense vegetation. A positive correlation between nest depth and water transparency has been confirmed.

Gill net sampling indicates white bass have sharply increased in Beaver and Bull Shoals reservoirs. Increases are attributed to a strong 1968 year class in both reservoirs--a year of high runoff and water levels. Walleyes also showed an increase in Bull Shoals reservoir with catches up from 0.22 fish/net day in 1968 to 0.91 fish/net day in 1969.

Midwater trawl sampling of shad populations in Beaver reservoir provided new information on growth and population size and revealed a strong positive correlation between shad abundance and white bass growth. Production of threadfin and gizzard shad in Beaver has exhibited great variation from year to year.

Cove rotenone sampling in Bull Shoals showed a decrease in total standing crop, due to a large decrease in adult gizzard shad. There was an increase in the intermediate and adult largemouth bass standing crop. Rotenone samples in Beaver indicated lower growth-rate and production of young-of-year largemouth bass in 1969, accompanied by a decrease in standing crop of intermediate and adult largemouth bass.

White bass eggs were hatched in jars and reared in aquaria to over 40 millimeters total length. Slight drops in temperature from incubation until feeding begins (10th day) are positively related to mortality.

Recaptures in Beaver of white bass from spawning ground tagging indicate a more complete distribution over the reservoir than in 1967-68. Slightly more than one-half of the tags recaptured by fishermen were voluntarily returned to us.

INTRODUCTION

A wet spring and a dry summer and fall occurred in 1969. Power generation outflows and evaporation lowered the Beaver Reservoir pool level 21.7 feet between February and December. The water level in Bull Shoals Reservoir dropped 23.9 feet in the same period.

POPULATION DYNAMICS

Gill net sampling

The annual January, 1969 gill net sample from Bull Shoals suggested a sharp increase in abundance of white bass. Catch per net day increased from 4.33 in 1968 to 9.51. This increase is attributed to a strong 1968 year class. The abundance of walleye has increased since 1967 when catch per net day was only 0.05 to 0.22 in 1968 and finally to 0.91 in 1969. White crappie had been absent in the sample for two years, but 0.38 per net day were taken in 1969. Abundance of gizzard shad, plains and highfin carpsuckers increased slightly in 1968, while other species exhibited no important changes.

White bass in Beaver increased from 0.73 in 1968 to 6.02 per net day in 1969, becoming the most abundant species in the sample. This increase was due to the strong 1968 year class. Except for a sharp decrease in 1967, white bass numbers have progressively increased through 5 years of sampling and now dominate the catch. With this change, the gill net catch composition in Beaver has become similar to that of Bull Shoals.

In the January 1969 sample, gizzard shad was the second most abundant species, with 2.62 captured per net day. Carp were third in abundance, but showed a decrease to less than one-half the 1968 catch. White crappie appeared for the first time in 1968 when 0.09 per net day were captured. In 1969, there was an increase to 0.16 per net day. Black crappie reached peak abundance in 1966, when 0.46 per net day were captured. They have progressively decreased since, with only 0.02 captured per net day this year. Numbers of walleye were greater than in 1968, but lower than in the three previous years.

Shad population estimation

Sampling shad populations with midwater trawls in both reservoirs continued. Population estimates were calculated for July through October in Beaver and July through September in Bull Shoals. Populations in both reservoirs were smaller than those of 1968.

Estimates on Beaver through 1966-1969 have been used to estimate annual production of 0-age shad. Production values have been computed for periods starting in the summer as soon as shad have grown large enough to be effectively captured in the midwater trawls and continued to the end of the year.

Production in Beaver during the 4-year period is shown graphically as the area under the curves in Figures 1 and 2. Estimates of production reveal enormous variation from year to year. Threadfin shad production in 1966 was 2.76 grams per square meter, 0.07 in 1967, 6.86 in 1968, and 1.09 in 1969.

Gizzard shad production in 1966 was 2.52 grams per square meter, too small to measure in 1967, then 4.39 in 1968, and 0.15 in 1969.

Comparison of white bass growth and shad production shows a direct relation. When shad production is high, white bass growth is good; when it is low, as in 1967, white bass growth is extremely poor.

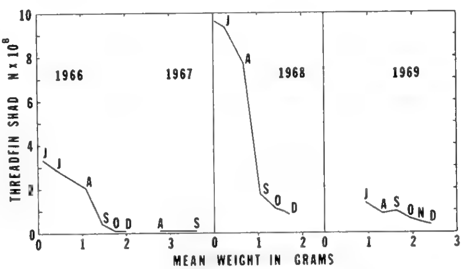


Figure 1.--Monthly changes in population numbers and average individual weights of 0-age threadfin shad in Beaver Reservoir, 1966-1969.

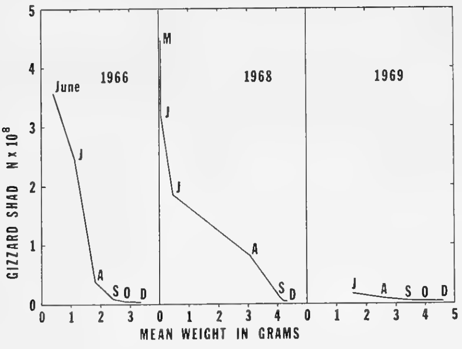


Figure 2.--Monthly changes in population numbers and average individual weights of 0-age gizzard shad in Beaver Reservoir, 1966-1969.

Cooperative study

Additional fish population studies using midwater trawls were conducted on Barren River and Nolin River Reservoirs in Kentucky in July. The study by the Kentucky Department of Fish and Wildlife Resources and Western Kentucky University under supervision of the Sport Fishing Institute is designed to demonstrate effects of high level and low level water releases on fish populations, fishing, plankton, benthos and water quality. Our midwater trawl sampling was to define vertical distribution of fish in the pelagic zones and estimate shad populations.

Alfred Houser

Cove rotenone sampling

The total standing crop estimate derived from 1969 cove rotenone sampling on Bull Shoals showed a decrease from 1968. This difference was due primarily to a decrease in adult gizzard shad. Increases were noted in the intermediate and adult largemouth bass population. The young-of-year threadfin shad crop was at its highest since 1966, equaling 6.7 lbs./acre. The carp standing crop also reached its highest since 1966. The standing crop of intermediate and adult channel catfish reached an all-time high since cove rotenone sampling was begun in Bull Shoals. Blue catfish were recovered for the first time since their introduction in 1967.

The reproduction or survival and growth of young-of-year largemouth bass was lower in 1969 than in 1968. While the growth rate of young spotted bass remained about the same, reproduction or survival slightly decreased. With the exception of the bluegill, young-of-year sunfishes reproduction or survival decreased. Intermediate and adult sunfish production also showed decreases from that of 1968.

Standing crop estimates from cove retonone samples in Beaver suggest slower growth and lower production of young-of-year largemouth bass than in 1968, and a decrease in intermediate and adult largemouth bass. Growth of young-of-year spotted bass was slightly faster, although production of young-of-year, intermediate and adults remained about the same. Intermediate and adult channel catfish decreased slightly from last year. With the exception of the bluegill, young-of-year sunfish production decreased. Intermediate and adult bluegill and longear sunfish increased and green sunfish decreased.

Young-of-year threadfin shad crops reached an all-time high in 1969 of 42.8 lbs./acre. The increase in total standing crop from 402.4 lbs./acre in 1968 to 444.4 lbs./acre in 1969 is attributed to larger adult gizzard shad and young-of-year threadfin shad crops.

Largemouth bass population estimates

Attempts were made in 1968 and 1969 to estimate the largemouth bass population in Beaver by the Schnabel mark and recapture method along 2.4 miles of a 75-acre cove shoreline. We used a boom-type shocker with a 230 volt a.c. generator to capture the fish, and a Floy FD-67 tag on fish over 200 mm. Fish less than 200 mm. were marked with a caudal fin clip. Eight nights of capture, mark and recapture began April 28 and ended May 15.

After 5 nights of tagging and recapture, 267 fish over 200 mm. were tagged and 24 percent recaptured, yielding an estimate of 672 (280/shoreline-mile). After 6 nights of shocking, 238 fish less than 200 mm. were marked and

24 percent recaptured, yielding an estimate of 868 (360/shoreline-mile).

In comparison, estimates from the same Beaver cove shoreline in May, 1968 showed 105 fewer bass greater than 200 mm. and 700 fewer bass less than 200 mm. The latter estimate provides dramatic evidence of the highly successful 1968 year class of largemouth bass associated with rising spring water levels and great numbers of young-of-year shad.

In Bull Shoals we selected a much larger cove of 395 acres with a shoreline of 8.9 miles to estimate the largemouth bass population by the Petersen method. This cove required 4 nights (April 7-10) of tagging and marking to cover the entire shoreline and 4 nights (April 14-21) of recovery of marked fish to estimate the population. As in Beaver, separate estimates were made of fish over and under 200 mm.

For fish over 200 mm., we tagged 283 fish and 23 percent recaptured, yielding an estimate of 1,174 fish (132/shoreline-mile). For fish less than 200 mm., 485 were marked and 21 percent recaptured, providing an estimate of 2,510 fish (237/shoreline-mile).

Considering the time involved per mile of shoreline and the percentage of recovery of marked fish, the Petersen method of estimating the population seems more practical than the Schnabel method. Shorter sampling periods should reduce errors introduced by extensive fish movement.

Age and growth of largemouth bass

In conjunction with the cove population estimates in 1968 and 1969 in both reservoirs, we collected largemouth bass scale samples for age and growth data. During 1969, we read 1,045 scales. The data were entered on IBM cards and a 7040 computer was used to calculate length-weight and body scale relations, and growth.

Horace E. Bryant

Exploratory sampling for young-of-year fish

Exploratory sampling continued into its second and final year. Objectives were to find (1) sampling techniques for age group 0 fish suitable for White River reservoirs; (2) distribution patterns for the major species; and (3) population estimation methods for young fish.

The 1969 effort concentrated on meter net sampling with a modified sampling design based on 1968 results. Intermittent collections began March 27, with a regular weekly sampling schedule from April 23 to July 24. Except for intermittent collections in the upper end of the reservoir and a reservoir-wide series made in one night, all sampling was in the Prairie Creek arm near the middle of Beaver reservoir. One night each week was spent sampling from the upper end of the Prairie Creek arm, the following night from the main channel at the mouth of Prairie Creek. Three samples were taken each night from each of the following: surface along shore, surface in mid-channel, 5 meters, 10 meters, and at 15 meters in the main channel area where depth permitted. Sampling sequence was random and hauls were standardized at 5 minutes each at 1500 rpm. Each haul filtered approximately 360 cubic meters of water at a towing speed of approximately 3.4 miles per hour.

Catch rates for all species varied considerably with date, depth, and even within replicates, but enough consistency was obtained for the major species taken to determine approximate spawning period, gross distribution patterns, early growth rates and an index of numbers present.

Species composition, dates captured and the date of highest mean density are shown in Table 1. Composition is similar to the 1968 meter net catch.

The spawning period was estimated for both shad species by back plotting growth curves. Shad prolarvae were not taken consistently in the Prairie Creek samples. The catch was apparently influenced by larvae drift into and out of the sampling area. Gizzard shad spawned from the second week of April to the third week

Table 1.--Composition, first and last dates captures, and week and mean density of highest catch of identifiable larval fishes captured by meter net in the Prairie Creek area of Beaver Reservoir, Arkansas, April 23 to July 24, 1969. Based on 120,796 fish from 357 hauls.

Species	Percent of total catch	Dates captured First	Dates captured Last	Maximum mean density Dates	Maximum mean density Number/1,000 m^3
Gizzard shad	46.7	4-30	7-24	5-14/15	1955
Threadfin shad	32.5	5-21	7-24	6-18/19	893
Lepomis, sp.	13.7	5-21*	7-24	6-18/19	464
Pomoxis sp.	5.3	4-30	7-24	5-14/15	247
Brook silversides	1.0	5-28*	7-24	7-16/17	31
White bass	0.5	4-30	6-19	5-14/15	25
Log perch	0.1	4-23	5-22	4-30/31	16
Micropterus sp.	t**	5-21	5-29	5-21/22	2
Carp	t	5-7	7-10	5-28/29, 6-11/12	t**
Channel catfish	t	6-18	7-24	6-18/19	t
Black bullhead	t	-	-	7-2/3 only	t
Flathead catfish	t	-	-	6-25/26 only	t

* Age group I + taken earlier; this is the first date larval were captured.

** Less than 0.1 percent of total catch, or less 1/1000 m^3. (1,000 m^3 equals 0.81 acre-foot.)

of June and threadfin from the first week of May to about July 1. This was a longer spawning period for both species than in 1968 and could be due to warming in the reservoir. Surface temperatures increased steadily and rapidly to 83° F. on May 29. Temperatures then dropped to 75° F. on July 5 and remained in the mid-70's throughout June but then increased rapidly into the 80's again about July 1.

We collected white bass eggs in the extreme upper end of Beaver on March 27 (the earliest sample taken) but the first prolarvae were not taken until April 10. Spawning continued through the third week of April. Crappie spawned from about mid-April until late June. Sunfish and brook silversides began spawning about mid-May and continued throughout the remainder of the sampling period.

Distribution patterns have not been determined for all species, but gizzard shad (Figure 3) seem to show a progressive seasonal change from concentration at the surface of both shoreline and mid-channel areas in late April and early May; to surface at the shoreline, and surface at mid-channel and 5-meter depths in mid-May; to the 5-meter depth for the remainder of the period. An exception to this pattern occurred on June 25 and 26, when numbers at 5 meters dropped drastically, but an increase was noted at the 15-meter depth. Several days of very high winds which completely mixed the upper 30 feet of water preceded this distribution shift.

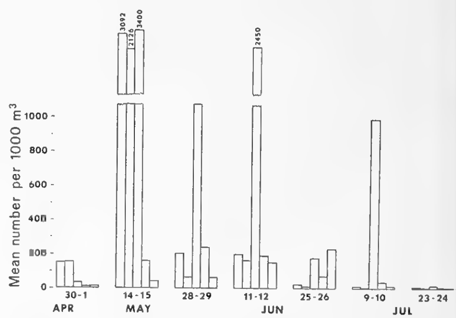


Figure 3.--Depth distribution of gizzard shad in the Prairie Creek area of Beaver Reservoir, by alternate weeks from April 30 to July 24, 1969. Mean number/1000 m^3 depict from left to right: surface along shore; mid-channel surface, 5 meters, 10 meters, and 15 meters.

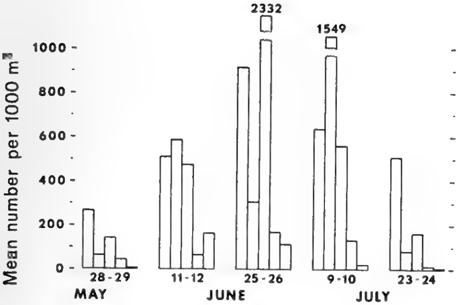


Figure 4.--Depth distribution of threadfin shad in the Prairie Creek area of Beaver Reservoir, by alternate weeks from May 28 to July 24, 1969. Mean number/1000 m^3 bars for each week depict from left to right: surface along shore; mid-channel surface, 5 meters, 10 meters, and 15 meters.

Threadfin shad occurred in greatest numbers in samples along the shoreline in May after which peak abundance alternated between the shoreline, surface and 5 meter samples for the remainder of the sampling period (Figure 4). Highest density occurred at the 5-meter depth on June 25-26. During the same period, there was a noticeable drop in the surface catch in mid-channel, due to movement caused by the wind and mixing conditions mentioned previously. The density at 10 and 15 meters was always less than at any of the shallower depths.

Analysis of variance of gizzard shad density and distribution showed significant differences between weeks and between depths but no difference between locations (main channel compared to tributary arm). There was interaction between week and location and between week and depth. Taking each week individually, and comparing locations by depth, significant differences were obtained only three weeks at the surface and at 5 meters. Comparing both the main channel and tributary arm, differences between depths were obtained in 7 of the 11 weeks.

Norval F. Netsch

Beaver Reservoir sport fish catch

Estimations of angler effort and harvest on Beaver Reservoir continued during the fifth year in cooperation with the Arkansas Game and Fish Commission and the U. S. Corps of Engineers.

From June 1968 - May 1969, anglers spent 521,600 hours, or 122,800 angler-days, harvesting about 461,900 fish weighing 518,700 pounds. This is a 17 percent increase in weight over the previous 12 months, even though estimated total effort was slightly less this year (Figure 5).

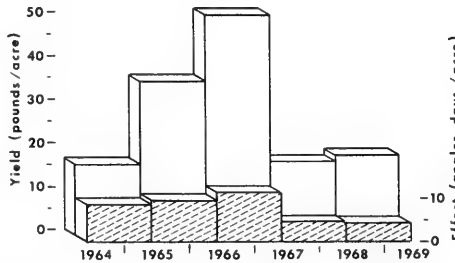


Figure 5.--Annual fishing effort in angler days per acre (front) and yield in pounds per acre (rear) on Beaver Reservoir, Arkansas, from June 1, 1964 through May 31, 1969. The reservoir was impounded in December, 1963.

Twenty-eight percent of total angler activity occurred from June through August, 13 percent during the fall, 2 percent during the winter, and 56 percent during the spring.

Yields of largemouth bass and crappies increased over the previous census year, but harvest of other species declined (Figure 6). During the white bass spawning run this spring many were caught in the upper reaches of the reservoir beyond the aerial count route and docks visited by the creel clerk. Estimates for white bass are therefore conservative.

Beaver Reservoir is following the same general pattern of yield reported by Missouri Conservation Commission biologists during the early years of Table Rock and Bull Shoals Reservoirs (Figures 7 and 8). Harvest estimates on Table Rock and Bull Shoals were conducted on

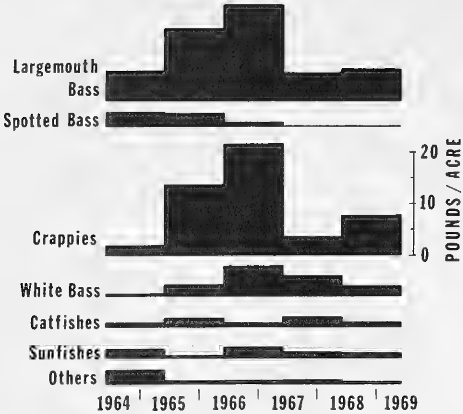


Figure 6.--Annual harvest of various sport fishes, in pounds per acre, from Beaver Reservoir, June 1964 - May 1969.

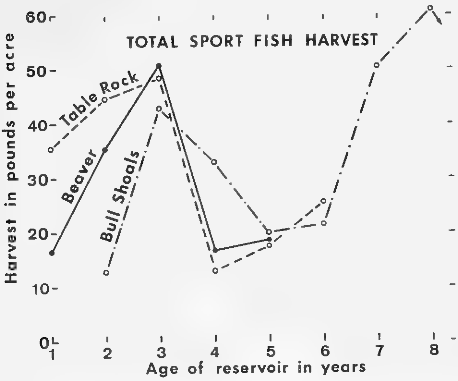


Figure 7.--Harvest estimates from Beaver Reservoir compared with adjusted harvest estimates (see text) from Bull Shoals and Table Rock Reservoirs during their early years of existence.

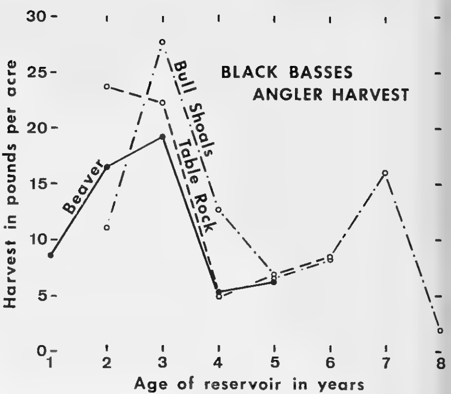


Figure 8.--Black bass harvest estimates from Beaver Reservoir compared with adjusted black bass harvest estimates (see text) from Bull Shoals and Table Rock Reservoirs during their early years.

selected major arms and upstream areas but adjusted to account for the fact that the censuses did not cover (Missouri D-J reports by Burress, Hanson, Funk and Fry). These areas typically have shallower mean depths, higher relative shoreline lengths, higher dissolved solids, and are more heavily fished than downstream, open water areas in Beaver. The proportion of the total harvest accounted for by the upstream census zone of Beaver (comprising one-fourth of the total reservoir area) has increased with age of impoundment. Factors used for converting previous harvest estimates in arms and upstream areas of Bull Shoals and Table Rock to total reservoir harvest per acre were drawn from the Beaver data as follows: first year of impoundment, 1.00; second year, 0.96; third year, 0.80; fourth and later years, 0.50.

David I. Morais

LIMNOLOGY

Physicochemical limnology

Monitoring of selected physical and chemical parameters continued on both impoundments. Sampling in Beaver was handled through contract with the University of Arkansas. Winter and spring water levels were well into flood pool on both reservoirs. Specific conductance and Secchi transparencies were generally lower than at comparable times in previous years, particularly during January and February. Water levels began falling in May and conductance and transparency readings generally increased throughout the remainder of the year.

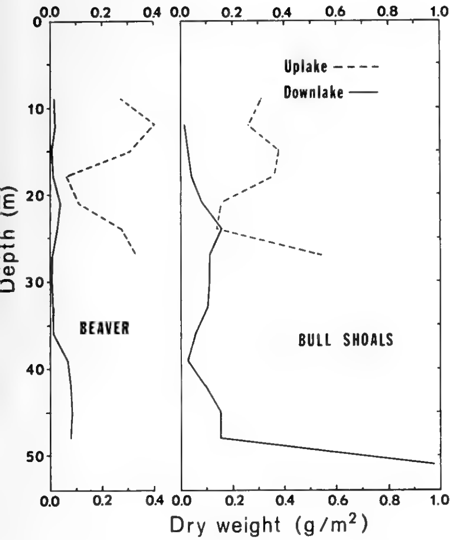


Figure 9.--Average profundal benthic biomass estimates of uplake and downlake transects on Beaver and Bull Shoals Reservoirs, as indicated by Petersen dredge collections, January-June, 1969.

Macroscopic profundal bottom fauna

Field collections were completed on a one year survey of the profundal benthos in Beaver and Bull Shoals. Comparisons of uplake and downlake regions of both impoundments were emphasized. Results from the January through June collections indicate a higher standing crop in the uplake region of both impoundments (Figure 9). A winter maximum in profundal benthic biomass appears typical for these impoundments.

The abundance of profundal organisms appeared to vary with the thickness of bottom sediments. Along the edges of the old river channel, and in areas with a steep sloping bottom, the benthos was poor. This may have involved sampling efficiency, as the lake bottom in these areas was often sufficiently compact to prevent dredges from biting deeply.

Immature stages of Chaoborus sp. and Chironomus attenuatus were abundant in the uplake region of both impoundments, but only meagerly represented in the downlake areas. Oligochaetes dominated collections from the downlake transects. Vertical movements were common among larvae of Chaoborus and C. attenuatus during the warmer portions of the year.

A comparison of the littoral macroscopic bottom fauna of Beaver and Bull Shoals reservoirs

Extreme structural heterogeneity in the littoral of both impoundments has produced rather severe sampling limitations. Conventional dredging has been ineffective. Shallow ceramic pots (12" inside diameter) filled with natural substrate and exposed for 28 days have been tested and used. Approximately 160 of these devices were set out during the late summer and 100 percent recovery was obtained using SCUBA.

These samplers have some limitations. Cold water imposes a seasonal SCUBA limitation which prevents 12-month studies. At best, field collections can be conducted 9 months of the year. SCUBA also limits the maximum depth at which these samplers can be used. However, the sampler appears well suited to comparative studies as it offers a sampling unit of remarkably uniform dimension and will function over a wide range of substrates. To date, studies involving ceramic pots have been designed to:

(1) Measure chironomid species diversity from selected littoral substrate as a measure of benthic community development and stability in impoundments of varying ages;

(2) Evaluate fish predation effects on the littoral benthic community;

(3) Monitor long-term changes associated with aging of White River impoundments.

Identification problems with some immature chironomids have delayed the analysis of benthos samples designed to measure chironomid species diversity. Samples from coarse rubble, gravel, sand, and silt have been collected. These are compared with regard to chironomid

Table 2.--Mean dry weight (g/m^2) of benthic biomass, and percent deviation from the control, of ceramic-pot collections in which fish were differentially excluded.

	Bull Shoals Reservoir		Beaver Reservoir	
	Weight	Percent deviation	Weight	Percent deviation
Control (no frame)	.105	-	.100	-
Frame only	.098	- 7	.151	+ 51
Frame with 1/4" mesh top	.060	-43	.067	- 33
Frame with 1/4" mesh excluder	.141	+34	.190	+ 90
Frame with 5/8" mesh excluder	.195	+84	.218	+117
Frame with 1" mesh excluder	.119	+13	.197	+ 97

species diversity, and general levels of benthos standing crop in each substrate.

Mass chironomid rearing was conducted at intervals throughout the year with approximately 30 associations having been obtained to date. This work will be continued on an expanded basis during the late winter and spring months of 1970. A chironomid reference collection is being assembled. When completed, this will include permanent slides of all life stages and keys to identification of reservoir species.

Tests designed to evaluate the effects of fish predation on littoral benthic organisms were conducted during the late summer. Small frames (16 x 16 x 12 inches), some of which were covered with various sized nylon net material, were used to exclude fish. These were placed over the ceramic pots buried in silt at water depths of 5 meters in both reservoirs and recovered after 28 days exposure. Structural barriers to fish predation resulted in an increase in benthos standing crop of near 100 percent in several instances (Table 2). An excluder of 5/8" bar mesh appeared most desirable in these tests. A one-inch mesh excluded sunfish over 70 mm. total length. Attempts to shade areas without providing protection from predation resulted in a decrease in benthic standing crop. Small sunfish were numerous in the study area of both impoundments. Shading provided protection for sunfish, and probably fostered increased predation. There was considerable evidence of carp predation in Beaver Reservoir. This may explain the increase in benthic standing crop where frames only were placed over sampling devices. Chironomids, Stenonema and Caenis increased in abundance where protection from predation was provided. Large burrowing forms, including Hexagenia and some oligochaetes, showed little difference, indicating selective predation. Additional attempts to evaluate predation effects are planned for 1970.

Littoral benthos samples were collected along transects during the late summer in the mid-lake regions of both impoundments. Standing crops of benthic invertebrates were highest near the shorelines and generally declined lakeward (Figure 10). In Bull Shoals, Stenonema and Caenis naiads were abundant at depths of 1-3 meters and declined rapidly lakeward. Naiads of Hexagenia were encountered wherever silty areas occurred within the littoral region. In Beaver Reservoir, the mayfly fauna was more poorly developed. Chironomids were the most abundant forms in both impoundments. However, the mean size of these organisms was small and some estimate of production rates will be needed before their contribution to the total benthos biomass can be assessed. In Bull Shoals Reservoir, chironomid abundance was highest near the shoreline (1 meter) and in the upper portion of the thermocline (9 meters). Chironomids were most abundant at 3 meters depth in Beaver Reservoir and reflected a rapid rate of shoreline erosion and shallow redeposition in this recently created impoundment. Chironomid abundance appeared to vary directly with the amount of siltation in Beaver Reservoir. Chaoborus larvae were more abundant in Beaver littoral samples than in Bull Shoals samples.

Larry R. Aggus

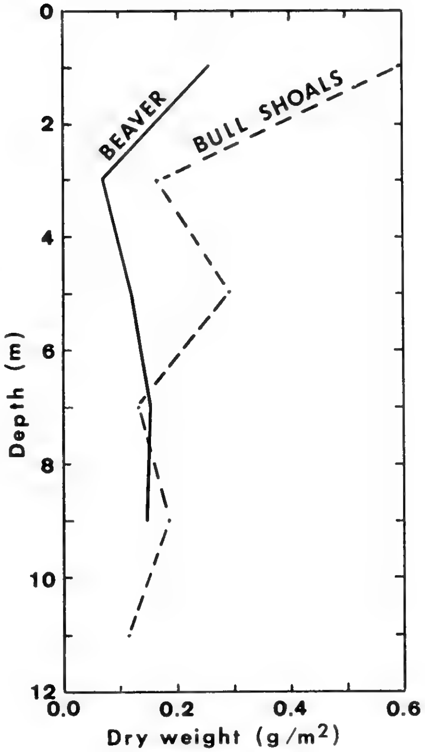


Figure 10.--Mean littoral benthic biomass estimates from transects in the mid-lake region of Beaver and Bull Shoals Reservoirs, as indicated by collections in ceramic pots, July-August, 1969.

LIFE HISTORY

Underwater studies

The weekly underwater SCUBA monitoring transects in Bull Shoals and Beaver which began experimentally in 1968 were expanded for the purpose of determining similarities and differences in reproductive requirements and spawning behavior of largemouth, smallmouth and spotted bass.

Observations in Bull Shoals began on April 15 when the water temperature reached 57° F. at 10 feet. Water level at the onset of spawning was approximately the same as in 1968 and 21 feet higher than when spawning began in 1967. As in 1968, no bass nesting was observed in the 800-yard steep bluff study area, a preferred spawning habitat of spotted bass in 1965, 1966, and 1967. Avoidance of bluff habitat for spawning by spotted bass is correlated with high water level, because during each of the 3 years of observation before 1968, water level at beginning of spawning was at least 15 feet lower. Nests of spotted bass in coves occurred at expected densities along with largemouth and smallmouth bass, suggesting that the increased cover available in coves where dense vegetation was inundated may have created an equally attractive spawning habitat.

Spotted bass were first observed spawning in Bull Shoals during 1969 on April 16 when the water temperature was 59° F. at nest depth. Largemouth and smallmouth bass nests were found one week later when the water temperature was 60° F. Reproductive activity continued through May for all three species with break-up of the last fry schools during the first week of June.

Four cove transects in Bull Shoals each week covered approximately 2,300 yards of habitat typical of the lower one-third of the reservoir. Within this shoreline distance, 53 fresh bass nests were found between April 16 and May 20. No preference among the three species for nesting substrate was apparent. However, there was some indication the species were selective

Table 3.--Numbers and depths of centrarchid nests encountered during weekly underwater transects of 2,300 yards of cove habitat in Bull Shoals during April and May, 1969.

	Number of nests	Nest depth range (feet)	Average nest depth (feet)
Largemouth bass	9	2.5- 5	4
Spotted bass	31	4 -13	6.5
Smallmouth bass	13	5 -10	7
White crappie	32	7 -15	10
Black crappie	6	5 -13	9

in nest depths (Table 3). These differences in nest depth preference may be minimal since water transparency was relatively low and nests may have been concentrated at unusually shallow levels. Data previously collected indicated greater depths are utilized for nesting when water transparence is high.

An 11-foot drop in water level in Bull Shoals during May appeared to adversely affect survival of fry schools. The densities of nests in egg and larval stages were similar to those observed in 1968, but the number of subsequent fry schools was much lower. Most guarded fry schools were observed for a shorter duration than in 1968, which suggests they may have suffered heavy mortalities from predation as the falling water forced them from cover.

In addition to observations of bass nesting, white and black crappie nests encountered during the cove transects were enumerated (Table 3). Black crappie nests had not been observed during the preceding four years.

Weekly underwater observations in Beaver Reservoir included two cove transects of 330 yards each and one of 320 yards on bluff habitat. Thirteen largemouth and 6 spotted bass nests were found in the cove transects while only 1 largemouth and 1 spotted bass nest were observed on the bluff between April 24 and May 29. No smallmouth bass or crappie nests were found.

Louis E. Vogele

Identification keys for larval and juvenile fishes

We were successful in artificial rearing of white bass to 45 millimeters. Before the stripping and fertilization operation on April 11, 1969, larvae had survived for only limited periods after hatching. Eggs were stripped and fertilized directly into a plastic hatching jar containing a shad tube and a piece of snug-fitting 2-inch thick treated matting. Neither bentonite nor rennet was used to separate eggs. The water used for incubation was filtered through fine gravel. The filter reduced the copepods to a low level, drastically reducing predation during the first four days after hatching.

Water temperatures in the hatchery system indicate larval white bass are sensitive to temperature drops of 5° F. (2.2° C.). Mortality attributed to temperature drops was estimated at 50 percent per lot, although the larger, more aggressive larvae appeared to survive.

Transportation of eggs from spawning grounds, where they are stripped and fertilized, to the experimental hatchery must be done by boat or vehicle. Neither is desirable due to vibration and its effect on eggs, even those which are water-hardened. The viable eggs were transported by boat on calm waters.

Feeding began between the seventh and tenth days when larvae assumed a horizontal swimming position (about 4.2 millimeters total length). Very finely ground, dry prepared meal (ground fines) was used initially. The larvae were observed to skim the surface a few moments after the food introduction, eating some of the finer particles. This meal was also introduced wet, but did not appear to be as acceptable. When the larvae reached about 4.5 millimeters total length (12th day) they were provided a small amount of natural food collected in a No. 10 mesh meter net. They readily accepted this food. Copepods present in the plankton catch were not observed to prey on the white bass larvae. Shad larvae were also taken in the tow and were eaten by white bass larvae. A few weeks later, larval shad captured by mid-water trawl were fed to the white bass. Many, however, were dead or dying and, although several were consumed, most were rejected. Later an attempt was made to feed young brook silversides to white bass; they were not eaten. It was our impression that the older larval shad and the brook silversides were too large to be acceptable. Larval carp were also fed to white bass and rejected.

Black and white crappie were hybridized and reared successfully. We agree with biologists of the Illinois Natural History Survey that hybrid crappie are more abundant in nature than previously believed. Characters are so similar that a hybrid parent may have been used in this experiment.

We obtained a few northern pike fry from the Arkansas Game and Fish Commission and reared them in our barge-mounted hatchery. When about 60 mm. long, these fish were fed two young carp (about 25 mm. total length) per day.

Studies of changes in morphometric characters of white bass during larval development were begun. A series of photomacrographs were taken of a specimen from each sample collected.

Movements of sport fishes in Beaver Reservoir

We began mark and recapture study of several species of sport fish in Beaver Reservoir in the fall of 1967. Trap nets and electro-fishing equipment, the principal gears used to capture fish for marking, were fished from fall to spring. Four trap nets were used in 1967-68 and six in 1968-69. The six trap nets in 1968-69 were fished in the mid-reservoir region, and in two major tributary arms of Beaver Reservoir. We used electro-fishing on the spawning grounds and in areas immediately below the spawning grounds. Hook and line fishing in early February several miles below the spawning grounds accounted for 106 white bass tagged, plus two recaptures from the other points of marking. A summary of mark and recapture results is presented in Table 4.

In 1968-69, over twice as many white bass were marked as in 1967-68. Trap nets accounted for 1,276 white bass in 1967-68 and 1,660 in 1968-69. Electro-fishing on the spawning grounds produced 342 white bass in early 1968 and 1,694 in early 1969. It is apparent that electro-fishing effort on the spawning grounds is the most productive method. Trap nets were fished 710 net-days and yielded 2.3 white bass/net day, while electro-fishing yielded 169 white bass/day. A trap net day is 24 hours of fishing per trap net while an average electro-fishing day is 4 hours. Trap nets produced 289 channel catfish, 77 flathead catfish, and 18 walleye during the 1968-69 period. Electro-fishing produced 10 walleye and 9 channel catfish during the spring of 1969.

White bass returns from the 1968-69 marking totaled 378 or 10.5 percent. Twenty white bass marked in the 1967-68 season were returned during the 1968-69 season, or 1.2 percent, for a total of 106 (6.6 percent) for fish tagged in 1967-68.

White bass marked from traps and recovered on the spawning grounds in 1969 had a median upstream migration of 0.21 miles per day, with extremes of 0.08 to 0.94 miles per day for 60 recaptures. For white bass marked on the spawning grounds and recovered downstream (beyond 5 miles and before July 15, 1969) the median distance was 0.50 miles per day, with extremes of 0.10 to 1.31 miles per day. One fish tagged in upper White River was caught 47 days later 44.5 miles down reservoir (0.94 miles/day) and within 3.5 miles of the dam. Six white bass were recovered by fishermen within 3 miles of the dam, while another 11 white bass were recaptured between 3 and 5 miles above the dam.

Table 4.--Summary of tagging results, 1967 to 1969

Year	Species	Number tagged	Number recaptured 1967-68	Percent return	Number recaptured 1968-69	Percent return	Total recaptured	Percent return
1967-68	White bass	1,618	86	5.3	20	1.2	106	6.6
	Channel catfish	192	11	5.7	0	-	11	5.7
	Flathead catfish	96	9	9.4	1	1.0	10	10.4
	Walleye	7	1	14.3	0	-	1	14.3
1968-69	White bass	3,589	-	-	378	10.5	-	-
	Channel catfish	298	-	-	29	9.7	-	-
	Flathead catfish	77	-	-	8	10.4	-	-
	Walleye	36	-	-	8	22.2	-	-

Table 5.--Growth of nine marked white bass with information on date and lengths at marking and recapture.

Date tagged	Days out	Total length (millimeters) at tagging	Total length (millimeters) at tagging	Growth in millimeters
1/19/68	461	225	303	78
1/9/68	457	250	315	65
1/3/68	419	255	325	70
2/23/68	388	245	330	85
1/15/68	330	264	327	63
4/9/69	271	322	330	8
12/23/68	180	257	269	12
2/7/69	164	340	345	5
1/27/69	54	210	213	3

Analysis recoveries by anglers without regard to size, shows a pattern of migration down the reservoir from the first of May to middle of August in 1969. Between March 29 to May 4, the heaviest recovery of tags came from the up-reservoir spawning grounds. Between May 10 to June 24, returns were heavy from the mid-reservoir region near the Highway 12 Bridge in the Prairie Creek arm area, while in mid- to late August a group of returns came from the vicinity of the mouth of Clifty Creek arm about 5 miles above the damsite. Fishing for white bass slacks off between mid-September and January.

Several white bass from the 1967-68 marking were recaptured by our gear during the 1968-69 season. Measurements taken indicate good growth of fish tagged with the Floy FD-67 tag (Table 5).

During the 1968-69 season we marked 298 channel catfish. There were 29 recaptures for a 9.7 percent return. The maximum distance traveled was 13 miles by a 336 mm. fish.

Trap nets caught a fairly large percentage of young channel catfish, many of which were too small to be tagged. Of the 298 marked, 196 (65.8 percent) were less than 375 mm. and 65 (21.8 percent) were less than 300 mm. total length. The median length was 370 mm. with extremes of 220 to 710 mm. No channel catfish marked in 1967-68 were recaptured during the 1968-69 season.

Fewer flathead catfish were marked in 1968-69. Of the 77 tagged, 8 (10.4 percent) were recaptured during the year. The median length was 750 mm. with extremes of 400 and 1,020 mm. The maximum distance of a recapture was 14.25 miles downstream from the point of tagging, 69 days after marking. This would be a minimum rate of 0.21 miles per day by the shortest route. One flathead catfish marked in April, 1968, was recovered 7-3/4 miles from the point of tagging after 494 days of freedom.

Five times more walleye were marked during 1968-69 than the previous year. Eighteen were marked from trap nets and an equal number from the spawning grounds. Nine of the spawning ground walleye marked were collected in gill nets or by electro-fishing by the Arkansas Game and Fish Commission. Their operations also recovered five walleye marked in our trap nets. Some white bass and walleye marked in trap nets fished about 2-1/2 miles above the confluence of the War Eagle Creek arm and White River arm were recovered on the War Eagle Creek spawning grounds. This indicates a homing tendency in these species in Beaver.

We frequently heard of fishermen who had caught marked fish but did not return the tags. Since little factual evidence was available, a series of tag recovery report forms were serially numbered and distributed in sporting goods stores, and other businesses frequented by fishermen. One location, a bait shop near the major white bass spawning ground on the White River, distributed many forms to fishermen with tagged fish in possession. Of the 51 serially numbered forms, 27 (52.9 percent) were returned to this office, and 24 (47.1 percent) were not returned. Only seven (1.4 percent) of 484 tagged white bass reported by anglers did not supply complete information.

The successful return of tags was attributed to heavy saturation of radio and newspaper media in northwest Arkansas, along with especially prepared posters in fluorescent red letters placed at docks. In addition, a 1/8 ounce doll fly was given in return for letters providing information on where and when tagged and distance traveled. When tags were returned to our office, information on the total white bass catch and number of tagged fish was requested. There was one tagged white bass per 36.9 fish caught, based on 14 tag recoveries from 517 white bass reported as total catch.

Thomas O. Duncan

CONTRACT RESEARCH

Food habits of the white and black crappie in Beaver Reservoir

A study comparing the food habits of the black and white crappie in Beaver Reservoir greater than 30 mm. in total length was initiated in June. From July to October, monthly samples were collected with an 8-foot midwater trawl and a few in gill nets. Beginning in November, larger crappie were captured with trap nets. Gill netting, trawling and electroshocking are being substituted for trap netting as necessary to obtain crappie during the winter and spring.

Analysis of the crappie stomachs completed to date indicates a strong preference of yearling and older crappie for young-of-year shad, particularly threadfin shad, from July through September. A comparison of the stomach contents of black crappie caught in July and August of 1965 with those caught during the same mon months of 1969 indicates a definite increase in the number of shad consumed, undoubtedly related to the greater number of threadfin shad present in Beaver in 1969. Young-of-year shad were the only fish found in the stomachs of the larger white crappie, and constituted the major food item by volume. Larvae and pupae of the phantom midge (Chaoborus) are the most numerous food organisms, but the volume of shad is much greater.

Robert L. Ball and Raj V. Kilambi

Limnetic zooplankton population dynamics

Monthly sampling of the zooplankton populations in Beaver and Bull Shoals Reservoirs began in August, 1969. Six stations on Beaver and seven stations on Bull Shoals were chosen for study. Collections were made with oblique tows of a Miller sampler from the surface to 50 feet.

Quantitative data are incomplete at this time as only 3 months of samples have been studied. In general, Bull Shoals has shown little change from an earlier 1964-1966 study. Beaver, now fully impounded, shows a higher zooplankton density at the downstream stations, which decreases in both diversity and density in the middle and upper reaches of the impoundment. This contradicts the results of an earlier 1964-1966 study, but could be only seasonal variation.

Dominant organisms in Bull Shoals Reservoir are the cyclopoid copepods, Bosmina, Daphnia, and the rotifers, Keratella and Asplanchna. In Beaver, the dominant cladoceran was Bosmina, with some Diaphanosoma and few Daphnia. Both çalanoid and cyclopoid copepods were present and Keratella and Asplanchna were the dominant rotifers.

Sam Damico and C. E. Hoffman

Beaver Reservoir physico-chemistry

Monitoring of selected physical and chemical parameters at 6 permanent study sites on Beaver Reservoir continued. Emphasis was placed on measuring nitrogen, phosphates, and total carbon for use in the prediction of eutrophication rates. Temperature, dissolved oxygen, transparency and conductance data were collected on a continuing basis. Bacteriological and phytoplankton sampling continued on a reduced scale during the last half of 1969.

A severe summer hypolimnetic oxygen deficit occurred in the uplake one-third of the reservoir. Water transparency in this region was generally low. Both dissolved oxygen concentration and water transparency increased in the downlake two-thirds of the reservoir.

High specific conductances were recorded in the uplake region during the late summer. Inflowing waters, primarily from the White River, remained in an identifiable mass which moved to the mid-lake area as a narrow density flow. The identity of this water was lost as thermal stratification began breaking up in October and November. Domestic pollution coupled with increased mineral concentrations as surface flows decreased, probably accounted for the high conductance of this stratum.

A summary report for the period July 1968 - June 1969 has been completed (M. S. thesis by Wayne Bennett). Most nutrient concentrations decreased during this period of study. Iron showed a 10-fold increase. However, multivariate analysis indicated no linear correlation between 15 chemical parameters.

Randall Bayliss and Robert A. Gearheart

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